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Cigarette Smoking and Electronic Cigarette Use among Malaysian Adolescents: Urgent Call for Action

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Editorial

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Adolescence is an important developmental period (Holliday & Gould, 2016; Yuan et al., 2015) characterised by engagement in risky behaviours, including the use of tobacco products such as cigarettes and electronic nicotine delivery systems such as e-cigarettes (e-cigs) (Casey et al., 2011).

The use of tobacco in the form of cigarettes and e-cigs is indeed of interest to the public health community and the nation at large. E-cigs are defined as devices that deliver aerosolised or vaporised nicotine form heating of liquids (e-juice) with constituents including nicotine, propylene glycol, glycerol, and other flavouring agents. It has been reported that 90% of smokers start smoking before the age of 18 years (Patel et al., 2017). The Tobacco & E-Cigarette Survey among Malaysian Adolescents (TECMA) 2016, a nationwide school-based survey, found 11.7% current cigarette smokers among students between 10 to 19 years old. 78.7% of ever cigarette smokers tried their first cigarette before the age of 14. In addition, 9.1% of the students were current e-cigarettes users, with 40.9% vaping once a day and 33.9% doing it 2 to 5 times per day. Alarmingly, data from the 2022 Adolescent Health Survey found a sharp increase in adolescent vaping prevalence among adolescents aged 13 to 17 years old, reaching a high of 14.9% in 2022 (Ministry of Health Malaysia, 2022).

E-cigs are heavily promoted directly to users include advertising and promotion at combustible cigarette point-of-sale (e.g., behind cashier's counter). E-cigs are also promoted via physical and online shops, internet, social media, events, etc. According to TECMA, 10.6% of school-going adolescents aged 10-19 years were offered a free trial session of e-cigarette/vape while 7.9% were offered a free e-cigarette/ vape liquid (e-liquid) (Institute of Public Health, 2016). With proliferation of e-cig promotions via the social media, internet, and vape shops (some under the guise of selling electronic products, handphones, etc.,), using celebrities and others, the number of dual users and vapers among non-smokers, especially adolescents in Malaysia can be even higher now.

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In early adolescence, development of executive function and neurocognitive processes in the brain has not fully matured. Adolescence is a sensitive period for maturation of brain circuits that regulate cognition and emotion, with resulting vulnerability to the effects of nicotine and tobacco. The rapidly changing, immature adolescent brain has differing sensitivity to drugs such as nicotine and tobacco, and drug exposure during this time can lead to long-term changes in neural circuitry and behaviour[6]. The American Academy of Paediatrics produced a policy statement showing evidence regarding the effects of nicotine on the developing brain. Nicotine has neurotoxic effects on the developing brain, an effect on the brain as a "gateway" drug for cocaine and other illicit drugs. The gateway theory postulates that smoking, especially among adolescence, increases the risk of substance use due to effects of nicotine, shown to be a neuroteratogen that exerts long-term, maturational effects at critical stages of brain development (Farber et al., 2015).

Nicotine is highly addictive and is the primary psychoactive component causing addiction. This is related to the high plasma concentration achieved and rapid nicotine delivery to the receptors in the brain from combustible cigarettes or e-cigs use. These two characteristics promote development of nicotine dependence and tobacco use disorder (TUD). The high concentration and rapid delivery of nicotine via smoking cigarettes or e-cigs result in release of neurotransmitters such as dopamine, norepinephrine, GABA, acetylcholine and serotonin. Having bursts of neurotransmitters release such as dopamine increases feeling of pleasure and general well-being. Similarly, smokers identify with the reduction in anxiety and tension with the release of GABA, acetylcholine and serotonin. However, these effects are short-lived due to the relatively short half-life of nicotine, i.e., about 2 hours. During this time, brain nicotinic acetylcholine (nAch) receptors are desensitized, and up-regulation of nAch receptors occurs. These processes contribute to the development of withdrawal syndrome, tolerance and craving for repeated use of nicotine, leading to tobacco use disorder (TUD) (American Psychiatric Association, 2013).

According to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) (APA, 2013), TUD is diagnosed when an individual uses tobacco for more than a year and a minimum of two of the eleven following sub-features appear:

1. More amounts of tobacco over a longer timeframe than planned are used
2. Inability to quit or lessen the amount of tobacco use in spite of efforts to do so.
3. Excessive amount of time spent on attaining or using tobacco.
4. Craving or strong desire or urge to use tobacco.
5. Relinquish responsibilities because of the tobacco use.
6. Use of tobacco persists in spite of its negative impacts both socially and in relationships.
7. Abandon career, social and other activities to use tobacco.
8. Use tobacco in harmful situations/settings.
9. Use is persistent even in the face of physical or emotional difficulties that are related to the use of tobacco.
10. Tolerance, as defined by either the need for markedly increased amounts of tobacco to achieve the desired effect or a markedly diminished effect with continued use of the same amount of tobacco.
11. Withdrawal, as manifested by either the characteristic withdrawal syndrome or the use of tobacco to relieve or avoid withdrawal symptoms.

In Malaysia, nicotine is regulated as a Group C poison under the Poisons Act 1952. However, exemption is given to tobacco products such as combustible cigarettes and cigars. Similar exemption was granted last year to nicotine in liquids and gels of e-cigarettes. However, there were no regulations approved prior to the removal, resulting in a judicial review against the then Health Minister. Following the standalone Control of Smoking Products for Public Health Act 2024, the regulations for tobacco products including e-cigs were supposed to be ready in June 2024 but yet to be released. This lacuna resulted in a significant increase in current vaping

among Malaysians 15 years old and older; from 0.8% in 2011 to 5.8% in 2023 (Institute for Public Health, 2024).

E-cig use is claimed as a safer alternative to combustible tobacco smoking. A recent review reported that nicotine e-cig probably help more people to stop smoking than NRT medications or nicotine-free e-cigs, with low numbers of unwanted effects. However, the authors warned that these results are based on a small number of studies in adults with wide variability in the measured data. More importantly, the reported unwanted effects are likely to change when more evidence becomes available (Hartmann-Boyce et al., 2020).

While some proponents of e-cigs are suggesting that e-cigs are useful for cessation of cigarette smoking, others have shown that e-cigs still contain harmful ingredients. The 2016 report of the U.S. Surgeon General on e-cigarette use among youth and young adults reiterates the fact that e-cigarettes can expose users to various chemicals, including carbonyl compounds, and volatile organic compounds, known to have adverse health effects. The health effects and potentially harmful doses of heated and aerosolized constituents of e-cigarette liquids, including solvents, flavourings, and toxicants, are not completely understood. In simple terms, e-cigarette aerosol is not harmless "water vapor" as claimed by some (U.S. Department of Health and Human Service, 2016).

Important strides have been made over the past several decades in reducing conventional cigarette smoking among youth and young adults especially in developed countries. Sadly, smoking prevalence among Malaysian adults, has remained at least 20% since the first National Health Morbidity Survey was conducted in 1986. The Ministry of Health in particular is trying its utmost to curb the smoking epidemic towards achieving the endgame in 2040 targeting smoking prevalence to be less than 5% (Ministry of Health Malaysia, 2021, National Strategic Plan on Tobacco Control 2015-2020). Hence, more stringent measures must be implemented so that whatever progress thus far will not be compromised by the initiation and use of new tobacco products, such as e-cigarettes or heated tobacco products. Effective implementation of the WHO Framework Convention on Tobacco Control as well the accompanying MPOWER strategies are needed to help protect Malaysians from the dangers of tobacco and nicotine, especially for our youths. The use of other nicotine products, including e-cigs is creating a new generation who are at risk of nicotine addiction and poisoning. We have failed to ban e-cigarettes and are now faced with the consequences of trying to regulate them, especially following the exemption of nicotine from e-cigarette liquids and gels by the previous Health Minister on 31st March 2023.

Various NGOs have come forward against cigarettes, e-cigs and other tobacco products which are targeting youth. Various platforms, including press conferences, social and printed media as well as reports to the authorities have been made. It is highly encouraging that the new standalone Tobacco Control Act 2024 is available, but sadly, the generational Endgame part has been dropped. Furthermore, regulations for the Act will be key, as better implementation and enforcement activities are urgently needed to thwart the vast promotional activities in Malaysia by both tobacco and vape industry. Hence, to protect young people from initiating or continuing the use of e-cigarettes and other tobacco products, stringent actions must be taken at the local, state and national levels. All parties must continue to take aggressive steps to protect our youth from the harmful effects of using tobacco products and e-cigarettes. These include enforcing effective taxation system and expanding smokefree areas, as well as regulatory authority over the manufacturing, distribution, and marketing. Ideally, banning e-cigarettes or any similar emerging products would be the best way to ensure our future generation will be free from nicotine addiction. The health and well-being of our nation's young people depend on it.

References

American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders: Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. Arlington, VA: American Psychiatric Association.

Casey, B., Jones, R. M. & Somerville, L. H. (2011). Braking and Accelerating of the Adolescent Brain. Journal of Research on Adolescent, 21, 21–33.

Farber, H. J., Walley, S. C., Groner J. A. & Nelson K. E. (2015). Section on Tobacco Control. Clinical Practice Policy to Protect Children from Tobacco, Nicotine, and Tobacco Smoke. Pediatrics, 136(5), 1008-17. https://doi.org/10.1542/peds.2015-3108.

Hartmann-Boyce J., McRobbie H., Lindson N., Bullen C., Begh R., Theodoulou A., Notley C., Rigotti N. A., Turner T., Butler A. R., Hajek P. (2020). Electronic cigarettes for smoking cessation. Cochrane Database of Systematic Reviews 2020, Issue 10. Art. No.: CD010216. https://doi.org/10.1002/14651858.CD010216.pub4.

Holliday, E. & Gould, T. J. (2016). Nicotine, Adolescence, and Stress: A Review of How Stress can Modulate the Negative Consequences of Adolescent Nicotine Abuse. Neuroscience and Biobehavioral Reviews, 65, 173–184.

Institute for Public Health (IPH). (2016). Tobacco & E-Cigarette Survey Among Malaysian Adolescents (TECMA) 2016. National Institutes of Health, Ministry of Health Malaysia, Kuala Lumpur. ISBN: 978-983-2387-30-5.

Institute for Public Health (IPH). (2024). National Health and Morbidity Survey 2023. National Institutes of Health, Ministry of Health Malaysia, Kuala Lumpur. ISBN: 978-967-5340-91-8.

Ministry of Health Malaysia. (2022). National Health and Morbidity Survey 2022. Adolescent Health Survey 2022. [Internet]. Available from: https://iku.gov.my/images/nhms-2022/Report_Malaysia_nhms_ahs_2022.pdf

National Strategic Plan on Tobacco Control 2015-2020. [Internet]. Available at: https://www.moh.gov.my/moh/resources/Penerbitan/Rujukan/NSP_Tobacco_buku_bind_24oct2015.pdf.

Patel, M., Kaufman, A., Hunt, Y. & Nebeling, L. (2017). Understanding the Relationship of Cigarette Smoking Trajectories Through Adolescence and Weight Status in Young Adulthood in the United States. Journal of Adolescent Health, 61, 163–170.

Poisons Act 1952. https://www.pharmacy.gov.my/v2/sites/default/files/document-upload/poisons-list-1.9.2020_0.pdf

U.S. Department of Health and Human Services. (2016). E-Cigarette Use Among Youth and Young Adults: A Report of the Surgeon General—Executive Summary. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Yuan, M., Cross, S. J., Loughlin, S. E. & Leslie, F. M. (2015). Nicotine and the Adolescent Brain. Journal of Physiology, 593, 3397–3412.

Journal of Pharmacy

Biological monitoring of iodine content in human breast milk over six months postpartum: A case study

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Abstract

Introduction: Iodine deficiency was commonly reported in infants and partly attributable to low breast milk iodine content. The role of iodine is crucial in preventing brain damage and hypothyroidism in infants. It is important to monitor the concentration of iodine in breast milk of postpartum mothers. This study aimed to validate an analytical method to determine iodine concentration in human breast milk for biomonitoring purposes. **Materials and method:** Expressed breast milk samples were collected several times a day throughout six months postpartum from a healthy lactating mother. Samples were prepared with nitric acid digestion and analysed by inductively coupled plasma mass-spectrometry. Data analysis was conducted using Microsoft Excel 2016 for assessment of validation parameters and longitudinal concentration of iodine. **Results:** The method validation parameters showed that linearity of calibration graph was 0.9987, limit of detection and limit of quantification were 0.218 µg/L and 0.661 µg/L, respectively. A recovery of 100.3% showed good accuracy, whereas inter-day and intra-day repeatability were 5.91% and 3.60%, respectively. The median iodine concentration was the highest in the first month (160.0 µg/L), then dropped to lower than recommended level (110 µg/L) from the second until six months postpartum (range: 31.9 - 98.7 µg/L). Fluctuation in median iodine concentration occurred over six months postpartum but circadian rhythm was observed to be consistent with "V" shaped curve pattern indicating higher concentration was exhibited in the morning and at night compared to evening. **Conclusion:** The analytical method was robust, accurate and reliable for measuring iodine concentration in human milk and applicable for biomonitoring. Deficiency in breast milk iodine content was observed in the second until six months postpartum. Iodine concentration in breast milk exhibited consistent circadian variation over six months postpartum.

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Introduction

In early life, infants primarily rely on breast milk as the main source of nutrients including iodine (Mosca & Giannì, 2017). Iodine is obtained from the dietary sources to produce thyroid hormone, thyroxine and triiodothyronine. Lack of iodine will consequently lead to poor neurodevelopment, cognitive impairment and mental retardation in children, and other medical conditions collectively termed as Iodine Deficiency Disease (IDD). IDD has been reported extensively worldwide and substantial reduction of infants' morbidity and mortality has been observed following iodine supplementation in various forms, for example in countries including Zaire, Papua New Guinea, Indonesia, China and Europe (Eastman & Zimmermann, 2018). Infants are particularly susceptible to IDD due to high iodine requirement for growth and development since they are born with very small amounts of iodine body reserve (Dror & Allen, 2018).

The monitoring of breast milk iodine concentration (BMIC) is important in infants who exclusively receive mothers' milk or donors' milk in the first six months of age. Iodine status in infants has been reported to be more accurately reflected by the maternal breast milk iodine status (Nazeri et al., 2018). It has also been demonstrated that higher BMIC at the second week postpartum was associated with a larger increase in infant growth and development compared to later stage (Ellsworth et al., 2020). BMIC is mainly affected by foods, supplements, or medications as it is not synthesised in the human body. Supplementation intervention has been demonstrated to slightly increase iodine status in postpartum women although not able to resolve deficiency status (Mulrine et al., 2001). Additionally, BMIC is also varied according to each breastfeeding session, either colostrum or later milk stages, between days postpartum and individuals (Ballard & Morrow, 2013).

Biological monitoring of iodine concentration in human milk is important for maintaining adequate amount of iodine requirement in breastfed infants. The standard monitoring of urinary iodine concentration does not usually reflect the BMIC (Huang et al., 2023). Literature has been conflicting addressing correlation of urinary iodine and BMIC. It was noted that limited monitoring was available of nutrition-sensitive data across the food system in Southeast Asia and Western Pacific Regions (Peters et al., 2023). A nationwide survey in 2010 indicated that Malaysia was at borderline adequacy despite absence of goitre endemic but urinary iodine level of most of the population was <100 μg/L (Selamat et al., 2010).

The lack of available data on the BMIC in Malaysian population could stem from limited access to analytical instruments and ethical considerations regarding the collection of human milk samples. ICP-MS is a highly sensitive instrument as it can detect metal and nonmetal elements in a sample at very low concentrations with extremely low detection limits up to ppt (parts per trillion) (Catenza & Donkor, 2022). The aim of this study was to develop and validate a method to quantify the amount of iodine present in human breast milk and ascertain its application in biomonitoring.

Materials and methods

Study participant

A postpartum woman was recruited to supply breast milk samples throughout the first six months postpartum. The participant was enrolled as a case study and was provided with a participant information sheet. She was informed about the study, and data collection sheet that included participant's relevant health information. Expressed breast milk (EBM) samples were provided in plastic containers labelled with date and time of which the milk samples were collected. The samples were collected throughout six months of lactation from June until November 2023. Samples were kept in a freezer at -21ºC until analysis at the Institute of Oceanography and Environment, University Malaysia Terengganu. The samples were analysed using ICP-MS with the conditions reported in the previously published study (Mohd-Taufek et al., 2023).

Sample preparation, blank preparation and wash solution

The milk samples, blank samples, and wash solution were prepared following the protocol that has been published previously (Mohd-Taufek et al., 2023). Sample preparation was done by mixing 1 mL of thawed milk with 9 mL nitric acid 1% (v/v) to form homogenous solution. The nitric acid 1% (v/v) was prepared by diluting 15.4 mL of nitric acid 65% (v/v) (Merck Suprapur) with deionised water up to 1 L. The blank samples were prepared for 10 sets using 10 mL nitric acid 1% (v/v) into a conical tube. The wash solutions between samples analysis were the Milli-Q water and acid washout was done for every 10 samples using the 1% (v/v) nitric acid.

Standards preparation

For standards preparation, a 1000 mg/L of iodide standard solution (Tracecert®) was used. Then, 0.1 mL of iodide standard solution was added into 50 mL of 1% (v/v) nitric acid in a polypropylene conical tube to prepare the standard stock solution. Six calibration standard solutions were then prepared by adding 1 mL of milk sample into 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL of iodide stock solution. Each standard solution was diluted to 10 mL with 1% (v/v) nitric acid, resulting in six solutions with final concentrations ranged from 0.02 μg/mL to 0.12 μg/mL.

Quality control

The certified reference material for human milk iodine is currently unavailable, and thus spiked samples were used to measure accuracy by using 1000 mg/L of iodide standard solution (Tracecert®). Then, 0.1 mL of single-element standard solution was diluted with 1% (v/v) nitric acid up to 50 ml for the purpose of standard stock solution. Then, 1 mL of each of five milk samples were spiked with 0.1 mL of 0.002 μg/mL iodine and made up to 10 mL by adding 1% (v/v) nitric acid. The validated method was then used to analyse all milk samples donated by a postpartum mother over six months postpartum. Spiked samples where known quantities of a substance added to a sample were freshly prepared daily. Analysis of study samples were run concurrently with the blanks, iodide standard solution (Tracecert®), and iodine-spiked milk samples. Samples were analysed using ICP-MS (Perkin-Elmer SCIEX model ELAN 9000) connected with DELL PC equipped with ELAN Instrument Control Session software (PerkinElmer Inc., Massachusetts, USA).

Sample analysis and analytical method validation

Sample analysis was conducted using the instrument and protocol that has been previously reported (Mohd-Taufek et al., 2023). Validation parameters assessments were reported based on linearity, Limit of Detection (LOD), Limit of Quantification (LOQ), accuracy and repeatability. A calibration graph of concentration *vs*. response was generated for final concentrations of 0.02 μg/mL, 0.04 μg/mL, 0.06 μg/mL, 0.08 μg/mL, 0.1 μg/mL, and 0.12 μg/mL.

Accuracy was assessed by comparing the result obtained from the analysis of spiked milk samples using 1000 mg/L of iodide standard solution (Tracecert®) calculated in the form of percent recovery.

Repeatability of the method was assessed on the same day (intra-day) using five spiked milk samples, and on three different days (inter-day), expressed as relative standard deviations (%RSD) and pooled relative standard deviation (RSDpooled) respectively.

Data analysis

Data on method validation parameters as well as breast milk iodine concentration were analysed using Microsoft Excel 2016. The collected samples were sorted into days, weeks, and months postpartum. Data were divided into three-time intervals which were 0400–1159, 1200–1959 and 2000–0359 to measure the variation over 24 hours according to the data and time stated for the milk samples. Data were analysed and reported descriptively.

Results

The calibration graph (Figure 1) for iodine over six concentration levels ranging from 0.02 μg/mL to 0.12 μg/mL was linear with correlation coefficient (r^2) value of 0.9987. The LOD and the LOQ were 0.218 μg/L and 0.661 μg/L respectively. The accuracy value that was calculated in the form of percent recovery

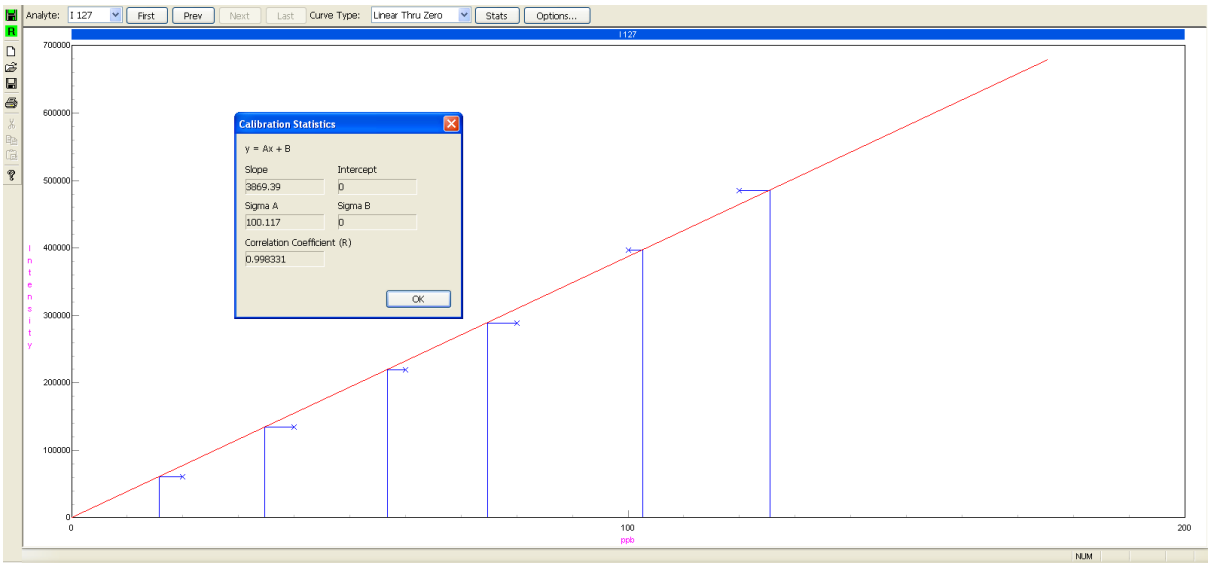

Figure 1: Calibration graph of concentration *vs.* response

Table 1: Recovery, inter-day (n=3), and intra-day repeatability of ICP-MS

Trace element	Unspiked concentration (µg/L)	Spiked concentration (µg/L)	Expected concentration (µg/L)	Recovery [%]	Repeatability% RSD (n=5)	
					Inter-day	Intra-day
I	11.5 ± 0.67	192.0 ± 4.53	180.5 ± 0.67	100.3 ± 2.70	5.91	3.60

Table 2: Concentration of iodine over six months postpartum

Trace element	mean/ median	Month postpartum					
		1	2	3	4	5	6
		n = 17	n =55	n = 44	n = 26	n =31	n = 18
I (µg/L)	mean ± SD	167.2 ± 69.0	45.8 ± 21.1	104.6 ± 41.3	39.6 ± 22.3	34.5 ± 16.2	39.8 ± 23.8
	median (range)	160.0 (72.9-276.0)	41.6 (11.3-96.8)	98.7 (30.8-215.0)	38.5 (7.2-115.0)	31.9 (9.4-70.2)	35.3 (10.4-85.7)

n: number of milk samples available

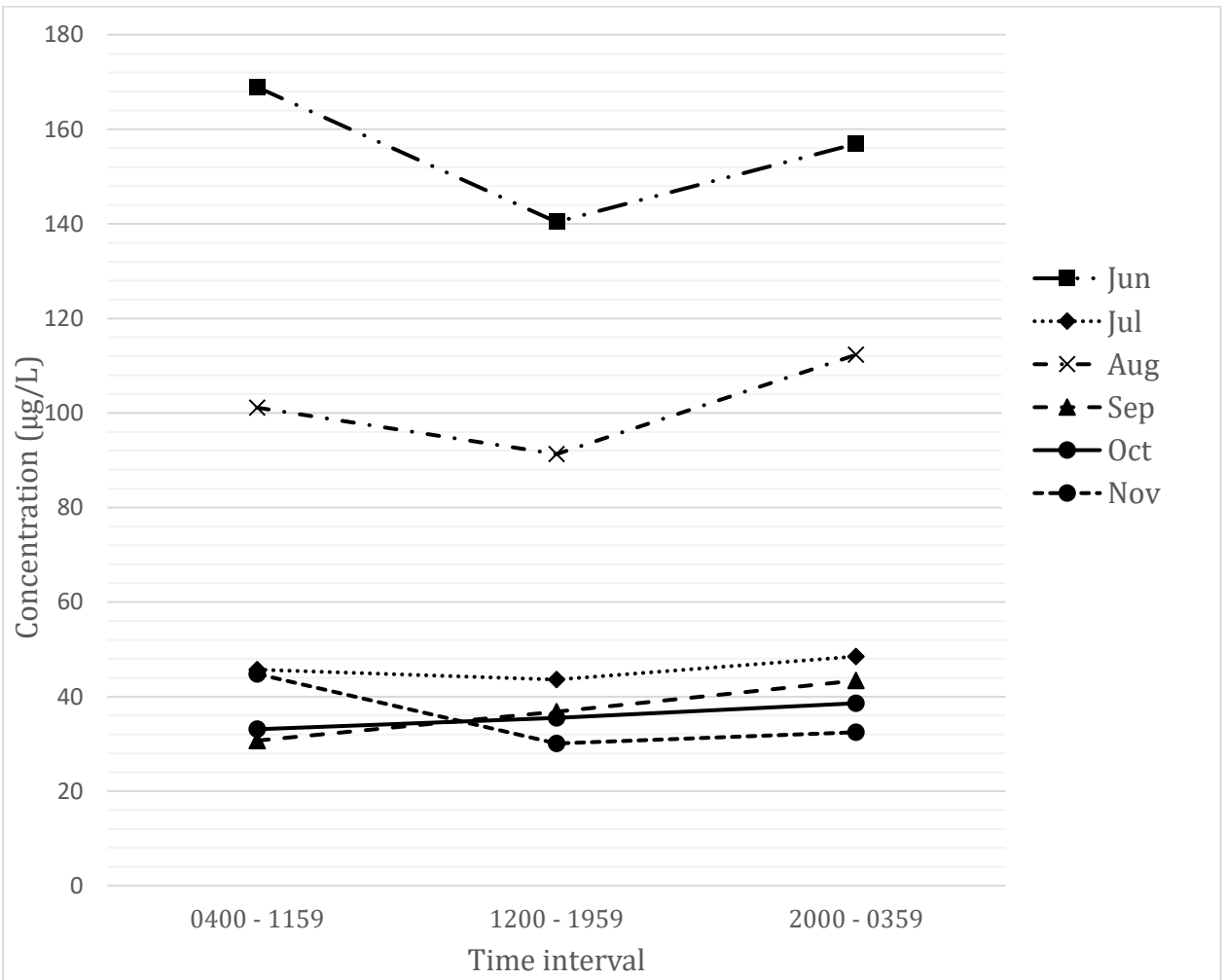

Figure 2: Median concentration of iodine (µg/L) in three-time intervals during 24-hour period over six months postpartum.

by using iodine-spiked milk samples was 100.3%. This finding indicated high recovery that eliminates the presence of matrix effect during sample analysis. The intra-day repeatability measured on the same day was 3.60%, and the inter-day repeatability measured on three different days was 5.91% (Table 1).

Application in biological monitoring of human milk iodine

The method was used to measure iodine concentration in 191 milk samples collected from a lactating mother over a period of six months. The participant was a 39-year-old Malay woman who had her first child, a male infant at 38 weeks of gestation by caesarean section, weighing 3.41 kg. The infant exhibited typical growth and development without any additional health issues apart from jaundice shortly after birth. Exclusive breastfeeding was maintained for the initial six months, followed by a combination of breastfeeding and introduction of solid foods. The mother claimed to include rice, fish, chicken, vegetables, fruits, and conventional supplements with zinc and iron in her dietary intake.

Table 2 showed the concentration of iodine over the period of six months. The highest median concentration of iodine was found in the first month postpartum (160.0 μg/L) then dropped to about a quarter (41.6 μg/L) in the second month. The subsequent months showed the fluctuations in the median concentration of iodine, but low concentration of less than 40 μg/L were observed in the fourth month onwards. Additionally, the widespread concentration range such as 7.2 – 115 μg/L in the fourth month showed similar trend in the fifth and sixth month.

Figure 2 demonstrated the circadian pattern of iodine concentration over six months postpartum. The median concentration of iodine throughout the day followed a 'V' shaped curve for all months except for the fourth (Sep) and fifth (Oct) month. The BMIC in the third month observed an improved median concentration but declined again in later stages, with the lowest BMIC being in the sixth month (Nov). Despite the circadian pattern being slightly different in the fourth, fifth and sixth month postpartum, the BMIC exhibited generally higher concentrations in the morning and at night, compared to afternoon/evening.

Discussions

The present study reports a validated analytical method measuring iodine concentration in human breast milk. The method exhibited strong linearity ($r^2 > 0.99$) with the recovery value within the acceptable range of 80-120%. The LOD and LOQ values indicated that the method was sensitive, whereas the inter-day and intra-day repeatability were considered precise as it falls below the limit of 20% based on the guideline of Codex Alimentarius Commission Joint FAO/WHO 2017. The method was robust, accurate and reliable for measuring iodine concentration in human breast milk and is applicable for biomonitoring of BMIC in population of lactating women.

Our study found that the median concentration of iodine in the first month postpartum was the highest at 160 μg/L, which met the requirement of Recommended Dietary Allowances (RDA) of 110 μg/day for infants up to six months of age. However, the median iodine concentration in the second month had declined below the adequate level, which was at 41.6 μg/L. The BMIC in the third month showed an increase in the median concentration of 98.7 μg/L, yet, still below the recommended level. Then, it further decreased to below 40 μg/L in later months postpartum. It could be noted that the lower and upper range for the fifth and sixth month fell below 100 μg/L. The low BMIC from the second month of lactation onwards could be due to insufficient dietary intake and supplementation of iodine in the postpartum mother. In Taiwan, it has been demonstrated that the estimated average BMIC was 111.6 μg/day (IQR: 78.3–172.1), which was sufficient for infants aged up to six months (Huang et al., 2023). However, global data highlighted that despite variation across populations, BMIC should be reaching a higher level of 150 μg/L in the first six months to ensure

infants would not be at risk of iodine deficiency (Dror & Allen, 2018). Our findings suggest that postpartum women and infants in Malaysia could be at risk of IDD if surveillance, prevention, and intervention strategies are not taken.

To address this issue, daily supplementation during lactation was proven effective in increasing BMIC following dose-response relationship. A study of donor human milk iodine concentration in Spain found that supplementation with iodine was associated with high BMIC of median (IQR) concentration of 148.5 (97.6 - 206.1) μg/L in 70% donors and thus considered as iodine-sufficient population (Ureta-Velasco et al., 2022). Although Malaysia has experienced a large decrease in incidence rate of IDD, it is still regarded as an area of iodine insufficiency (Wei et al., 2023). Therefore, our findings suggest that monitoring of BMIC in lactating women population is crucial to establish iodine sufficiency status in vulnerable populations of postpartum mothers and their infants. Data on BMIC will be able to indicate iodine supplementation in addition to iodine enriched food products to improve clinical outcomes in infants.

The circadian rhythm of BMIC in our study was reported following three different time intervals. There was a consistent pattern on iodine concentration throughout the day where lower concentrations were found in the afternoon/evening compared to the morning concentration and at night. This finding was consistent with other studies conducted in Northen China where the circadian rhythm exhibited a distinct "V" pattern over 24 hours (Zhang et al., 2023). The same study also reported that iodine concentration in breast milk significantly correlated with dietary iodine intake of the postpartum woman. We speculate that the utilisation and metabolism of iodine in the body may follow specific diurnal pattern based on the need to regulate the circadian rhythm of the infants.

The limitation of our study includes the lack data on the iodine intake of the postpartum woman and findings from one participant does not reflect a true representation of iodine status in Malaysia population. However, our findings found a case of poor iodine status of a postpartum woman in the first six months of postpartum that indicate the needs of BMIC monitoring in Malaysia population. Women living in regions impacted by iodine deficiency, who exclusively breastfeed their babies, should consider incorporating a supplement containing at least 200 μg of iodide per day, following the RDA.

Conclusion

The analytical method was robust, accurate and reliable for measuring concentration of iodine in human milk and applicable for monitoring BMIC in lactating women to prevent IDD in infants. The BMIC in the first month postpartum met the RDA requirement needed by infants but iodine insufficiency occurred and BMIC decreased over lactation stages. The circadian variation of "V" shaped curve of BMIC was observed. Future studies with larger sample size are required to assess BMIC in Malaysia.

Authors contributions

N.H.M.T., A.S.M.S., and J. B. conceptualised and planned the experiment, oversaw sample and data collection, conducted data analysis, while A.Z.A. analysed the samples and data. All authors contributed to the writing of the manuscript.

Ethical Approval Statement

Ethics approval was obtained from IIUM Research Ethics Committee with the project ID IREC 2021-053.

Informed Consent Statement

Informed consent was received from the study participant.

Conflict of interest

There is no conflict of interest for all authors.

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References

Ballard, O., & Morrow, A. L. (2013). Human Milk Composition: Nutrients and Bioactive Factors. Pediatric Clinics of North America, 60(1),49–74. https://doi.org/https://doi.org/10.1016/j.pcl.2012.10.002

Catenza, K. F., & Donkor, K. K. (2022). Determination of Heavy Metals in Cannabinoid- Based Food Products Using Microwave-Assisted Digestion and ICP-MS. Food Analytical Methods, 15(9), 2537–2546. https://doi.org/10.1007/s12161-022-02315-1

Dror, D. K., & Allen, L. H. (2018). Iodine in human milk: A systematic review. Advances in Nutrition, 9(10), 347S-357S. https://doi.org/10.1093/advances/nmy020

Eastman CJ, Zimmermann MB. (2018). The Iodine Deficiency Disorders. [Updated 2018 Feb 6]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000- . Available from: https://www.ncbi.nlm.nih.gov/books/NBK285556/

Ellsworth, L., McCaffery, H., Harman, E., Abbott, J., & Gregg, B. (2020). Breast Milk Iodine Concentration Is Associated with Infant Growth, Independent of Maternal Weight. Nutrients, 12(2), 358. https://doi.org/10.3390/nu12020358

Fisher, W., Wang, J., George, N. I., Gearhart, J. M., & McLanahan, E. D. (2016). Dietary iodine sufficiency and moderate insufficiency in the lactating mother and nursing infant: A computational perspective. PLoS ONE, 11(3),1–25. https://doi.org/10.1371/journal.pone.0149300

Huang, C.-J., Li, J.-Z., Hwu, C.-M., Chen, H.-S., Wang, F.-F., Yeh, C.-C., Yang, C.-C. (2023). Iodine Concentration in the Breast Milk and Urine as Biomarkers of Iodine Nutritional Status of Lactating Women and Breastfed Infants in Taiwan. Nutrients, 15:4125. https://doi.org/10.3390/nu15194125)

Mohd-Taufek, N. H., Mohmad Sabere, A. S., Mohamad Jamahari, U. S., Amran, N. B., Fata Nahas, A. R., & Bidai, J. (2023). Determination of Zinc, Copper, Selenium, and Manganese in Human Milk using Acid Digestion by ICP-MS and its Application in Biological Trace Element Monitoring. Journal of Pharmacy, 3(2), 129-139

Mosca, F., & Giannì, M. L. (2017). Human milk: composition and health benefits. Pediatria Medica e Chirurgica, 39(2). https://doi.org/10.4081/PMC.2017.155

Mulrine, H. M., Skeaff, S. A., Ferguson, E. L., Gray, A. R., & Valeix, P. (2010). Breast-milk iodine concentration declines over the first 6 mo postpartum in iodine-deficient women. American Journal of Clinical Nutrition, 92(4), 849–856. https://doi.org/10.3945/ajcn.2010.29630

Nazeri, P., Dalili, H., Mehrabi, Y., Hedayati, M., Mirmiran, P., & Azizi, F. (2018). Breast Milk Iodine Concentration Rather

than Maternal Urinary Iodine Is a Reliable Indicator for Monitoring Iodine Status of Breastfed Neonates. Biological Trace Element Research, 185(1), 71–77. https://doi.org/10.1007/s12011-018-1246-9

Peters, R., Li, B., Swinburn, B., Allender, S., He, Z., Lim, S. Y., Chea, M., Ding, G., Zhou, W., Keonakhone, P., Vongxay, M., Khamphanthong, S., Selamat, R., Dayanghirang, A., Abella, E., Da Costa, F., Chotivichien, S., Ungkanavin, N., Truong, M. T., Nguyen, S. D., … Poh, B. K. (2023). National nutrition surveillance programmes in 18 countries in South-East Asia and Western Pacific Regions: a systematic scoping review. Bulletin of the World Health Organization, 101(11), 690–706F. https://doi.org/10.2471/BLT.23.289973

Selamat, R., Mohamud, W. N., Zainuddin, A. A., Rahim, N. S., Ghaffar, S. A., & Aris, T. (2010). Iodine deficiency status and iodised salt consumption in Malaysia: findings from a national iodine deficiency disorders survey. Asia Pacific journal of clinical nutrition, 19(4), 578–585.

Ureta-Velasco, N., Keller, K., Escuder-Vieco, D., Serrano, J. C. E., García-Lara, N. R., & Pallás-Alonso, C. R. (2022). Assessment of Iodine Concentration in Human Milk from Donors: Implications for Preterm Infants. Nutrients, 14(20), 1– 14. https://doi.org/10.3390/nu14204304

Wei, R., Wang, Z., Zhang, X., Wang, X., Xu, Y., & Li, Q. (2023). Burden and trends of iodine deficiency in Asia from 1990 to 2019. Public Health, 222, 75 – 84. https://doi.org/10.1016/j.puhe.2023.06.034

Zhang, Y., Zhao, X., Shan, L., Jia, X., Liu, J., Gu, W., Zhang, Z., Zhang, X., & Sang, Z. (2023). Variations in Breast Milk Iodine Concentration over 24 h among Lactating Women in Northern China. Journal of Nutrition, 153(1), 208 – 214. https://doi.org/10.1016/j.tjnut.2022.11.024

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The Investigation of Phytochemicals and Antioxidant Properties of *Champereia Manillana* (Blume) Merr Stem Bark

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Abstract

Introduction: *Champereia manillana* (Blume) Merr. is one of the plant species that lacks research despite many beneficial claims from consumers. Studies conducted on this species have merely discussed bioactive phytochemicals in the leaves and roots. Therefore, the objectives of this study are to screen for terpenoids, triterpenoids, diterpenoids, carotenoids, flavonoids, phenolics, and steroids, and to evaluate the antioxidant activities of *C. manillana* stem bark methanolic extract. **Methods:** The plant material was collected from the Forest of 'Ilm, IIUM. The stem bark powder was macerated in methanol. Phytochemical screening tests were utilized to determine the presence of the phytochemicals. Thin-layer chromatography (TLC) analysis was carried out on the samples using iodine vapor, ferric chloride solution, vanillin solution, and UV light. Next, the total phenolic and flavonoid content tests were conducted to obtain quantitative results, and the antioxidant activity was assessed using the DPPH assay. **Results:** *C. manillana* stem bark extract tested positive for steroid and terpenoid contents and negative for carotenoids and flavonoids. For the TLC, the extract was found positive for iodine vapor, UV light, and vanillin/H2SO4 and negative for the ferric chloride test. The flavonoid content was 0.995 mg CE/g, while the phenolic content was 12.326 mg GAE/g. For the DPPH assay, the IC_{50} value was high (26 mg/mL) compared to the positive control, ascorbic acid, which had an IC_{50} value of 6.730 µg/mL. The percentage inhibition at 10 mg/mL was 23.4%. **Conclusion:** Phytochemical screening indicates the presence of steroids and terpenoids in *C. manillana* stem bark methanolic extract. Compared to the standard (ascorbic acid), the antioxidant activity of the extract is very weak.

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Introduction

The usage of herbs and plants in the world community these days is very common, whether it is for traditional medicine, modern medicine, or cooking recipes. Based on certain beliefs, raw herbs can heal illnesses better than modern medicines. However, to prove this statement, many factors must be considered, such as hygiene and the active ingredient content within the raw herbs. Our older generation, especially from Asian countries, loves to consume herbs as part of their meals and holds a strong belief that herbs keep them healthy during old age (Musa et al., 2022). The ease with which herb plants can be grown and harvested might be the main reason why they are so commonly consumed by past generations (Welz, Emberger-Klein & Menrad, 2018). Since the use of herbs usually relies on traditional knowledge passed down from previous generations, the exact composition of the phytochemical content within the herbs remains unknown until modern medicinal technologies make discoveries (Musa et al., 2022). In recent years, the study of herbs and plants has increased significantly, aiming to achieve a better understanding of their pharmacological importance and potential health effects. Hence, to achieve a complete understanding of a plant's safety, toxicology, and compounds, proper and rigorous investigations need to be conducted.

In Malaysia, one of the famous herbs is *C. manillana (Blume) Merr.,* locally known as *'pucuk cemperai'*. It is a monotypic genus from the Opiliaceae family of plants and can be widely found from the Andaman Islands to Papua New Guinea, including other countries such as Thailand, Vietnam, Taiwan, China, Singapore, Java Island, and many more (K. Jeyaprakash & N. Balachandran, 2018). *C. manillana* is a small tree that can grow up to 20 meters tall in lowland tropical rainforests at altitudes up to 1,600 meters. It has a variety of stalk leaves, including oblong, oval, and lance shapes, while both sides of the leaf blades are hairless (Yang et al., 2017). This species can be easily found in Pahang, and the locals commonly use it to make a dish known as *masak lemak pucuk cemperai*. This species is claimed to contain a high concentration of antioxidants that help protect the body against heart disease and cancer and boost the immune system. Additionally, some people use the pounded root of *C. manillana* to make a poultice for ulcers, while the boiled roots are used for healing rheumatism. Possible extinction is not an issue for *C. manillana* because this species is still widely distributed in our country and can easily be found in any rainforest (Denny, Marfuah Wardani, & Adi Susilo, 2021).

Fig. 1: *Champereia manillana* from the Forest of 'Ilm IIUM

Even though this plant has not received proper attention in research and studies, the leaves of *C. manillana* have been claimed to contain many beneficial phytochemical compounds that provide antioxidant activity, such as squalene, β-carotene, dietary lutein, and phytol (K. Jeyaprakash & N. Balachandran, 2018). Squalene is a natural triterpene with molecular properties directly linked to its activity against free radicals, acting as a free radical scavenger or quencher in certain cases. With its antioxidant properties, some studies have reported that squalene can suppress advanced colon cancer formation by preventing crypt multiplicity inside the intestine (Micera et al., 2020). The second phytochemical component found in *C. manillana* is dietary lutein. Lutein is a fat-soluble carotenoid phytochemical with several pharmacological properties (Rahman et al., 2023). Lutein has antioxidant, anti-inflammatory, anticancer, cardioprotective, and eye-protective activities. It exerts its ability to prevent the rapid growth of tumors and induce cancer cell apoptosis by inhibiting angiogenesis in mammary gland tumors. Another component is β-carotene, a carotenoid group compound (terpenoid) that serves as pigmentation in the plant kingdom. Its structure consists of a conjugated double bond with a retinyl group at both ends. β-carotene is the major precursor of vitamin A and contains many therapeutic properties for humans (Bogacz-

Radomska & Harasym, 2018). Lastly, the phytol component found in *C. manillana* is identified as Acyl-CoA cholesterol acyltransferase (ACAT). Phytol is a cyclic diterpene in the plant that forms part of the structure of chlorophyll. Nowadays, it is common to include phytol in pharmaceutical products due to its antinociceptive effect, which reduces pain by blocking the pain sensory neuron (Carvalho et al., 2020). Based on previous research related to the beneficial properties of *C. manillana* leaves, we aim to explore the phytochemicals and antioxidant activity of the stem bark.

Materials and methods

Plant collection and preparation

250 g of fresh *C.manillana* stem bark (Voucher Specimen : PIIUM 0357) (Species verification by Dr Shamsul Khamis from UKMB herbarium). A voucher specimen deposited at Kulliyyah of Pharmacy IIUM was collected from the Forest of 'Ilm IIUM Kuantan on 15 October 2023. The stem bark was rinsed and dried in the oven for two (2) days with a temperature range from 40-60 ℃ (Abubakar & Haque, 2020). Next, the dried stem bark was processed into powder form using an electric grinder.

Plant extraction

150 g of the fine powder was taken to be macerated with 950 mL of methanol in the conical flask for 5 days at room temperature (Abubakar & Haque, 2020). The extract solution was filtered using filter paper and the methanol was removed using a rotary evaporator. The crude methanol extract was kept for the next usage. The percentage yield is calculated following formula (1) as below:

$$\text{Percentage yield (\%)} = \frac{\text{mass of crude methanol}}{\text{mass of sample}} \times 100 \quad (1)$$

Methanol was chosen for the extraction process due to its superior ability to extract a wide range of phytochemicals compared to ethanol. Research has shown that methanol often provides higher extraction yields of total phenolic content (TPC) and is effective at extracting various other bioactive compounds. Specifically, methanol has been reported to achieve the highest extraction yields of TPC, the second highest extraction of total flavonoid content (TFC), and the highest yield of extractable solids (Goltz et al., 2012).

Phytochemical screening

A phytochemical test is a qualitative test that indicates the absence or presence of the targeted compound. The procedures below were referred to in various articles with minor modifications.

Terpenoid Test

For the Terpenoid Test, 50 mg of the extract was weighed and dissolved in 2 mL of dichloromethane. The solution was filtered, and then 2 mL of concentrated sulphuric acid (H_2SO_4) was added to the test tube. The formation of a reddish-brown coloration at the interface indicated the presence of terpenoids (Dubale et al.,2023).

Steroid Test

For the Steroid Test, 50 mg of the extract was weighed and dissolved in 2 mL of dichloromethane. After filtration, a few drops of acetic acid were added to the sample, and the mixture was boiled in a water bath for 10 minutes. The sample was rapidly cooled using an ice bath and then 2 mL of concentrated sulphuric acid (H_2SO_4) was added. Any formation of brown ring at the junction indicated that the presence of steroid (Dubale et al., 2023).

Carotenoid Test

In the Carotenoid Test, 100 mg of the extract was dissolved in 10 mL of chloroform and filtered. A few drops of concentrated sulphuric acid (H_2SO_4) were added to the test tube containing the filtered solution. A blue color at the interface showed the presence of carotenoids (Ajayi & Ajibade O, 2011)

The Carotenoid Test utilized 100 mg of extract, double the amount used in other phytochemical tests, to ensure sufficient sensitivity and accuracy in detecting carotenoids. Carotenoids are often present in lower concentrations compared to other phytochemicals like terpenoids, steroids, and flavonoids. By using a larger quantity of the extract, the test increases the likelihood of detecting carotenoids even if they are present in small amounts. This approach enhances the test's reliability and reduces the risk of false negatives, ensuring that the presence of carotenoids can be accurately confirmed (Goltz et al., 2012).

Thin layer chromatography (TLC)

Thin-layer chromatography (TLC) facilitates the separation and identification of various components within a mixture, relying on the principles of adsorption and capillary action. The sample is applied to one end of the TLC plate and then the

plate is positioned vertically inside a sealed chamber containing an organic solvent (mobile phase). Capillary action causes the mobile phase to ascend the plate, and components of the sample migrate different distances based on their interactions with the stationary phase (the TLC plate) and the mobile phase. Once the solvent reaches the top of the plate, it is taken out of the chamber and allowed to dry (Qin et al., 2021). For this method, the TLC silica gel plate 60F254 (Merck, Germany) was cut into 10 cm x 10 cm in dimension. The 10 mg of sample was dissolved in 1 mL of methanol. The dissolved sample was applied to the TLC plate using a capillary tube as 1 cm band. Next, the plate was placed inside the TLC-developing container that consisted of ethyl acetate and methanol with a ratio of 8:2 for 10 minutes, following the method by Ameerah Shaeroun et al., (2019).

Phytochemical analysis of the TLC for methanol extract

UV Lamp Analysis

The TLC plate was viewed under UV light which the wavelengths 254 and 366 nm in a dark room and the presence of aromatic compounds was seen through black and fluorescence colour on the TLC plate (chromatogram) (Ameerah Shaeroun et al., 2019).

Iodine Vapour

The TLC plate was sprayed with iodine vapour to detect the presence of a compound with double bond linkages. (Ameerah Shaeroun et al., 2019)

Ferric Chloride

The TLC plate was sprayed with ferric chloride solution to detect the presence of phenolic compounds. (Ameerah Shaeroun et al., 2019)

Vanillin/ H_2SO_4

The TLC plate was sprayed with vanillin/ H_2SO_4 solution to detect the presence of terpenoid compounds. (Ameerah Shaeroun et al., 2019).

Total phenolic content test (TPC)

TPC for the standard gallic acid and the methanol crude were performed following the method by Herald et al. (2012) with minor modification.

TPC for standard gallic acid

The stock solution of gallic acid was prepared by two-fold serial dilution in distilled water, resulting in eight different concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781 µg/mL. Each of these concentrations was then used to prepare the standards for the assay. Specifically, 20 µL of each stock solution was transferred into a 96-well plate in triplicate. To each standard well, 100 µL of Folin-Ciocalteu reagent and 80 µL of sodium carbonate were added, making a total volume of 200 µL per well. In another set of wells, the blank consisted of 20 µL of the standard solution, 100 µL of distilled water, and 80 µL of sodium carbonate. The blank was also prepared in triplicate. Finally, the well plate was incubated for 2 hours in a dark room before measuring the absorbance using a microplate reader at 750 nm (Herald et al., 2012).

Estimation of TPC for sample extract

The 15 mg sample solution was prepared into two concentrations which were 5000 ug/mL and 10000 ug/ml. These concentrations were chosen to ensure that the phenolic content could be detected even at low concentrations, which is crucial for accurately assessing the phenolic profile of the extract. Using higher volumes helps to amplify the detection signal, thereby enhancing the sensitivity of the assay (Simčič, Stibilj, & Holcman, 2011). Using larger sample volumes in phenolic content assays has been advocated in research because it enhances the reliability of detecting phenolic compounds, particularly when they are present in trace amounts. This method ensures a comprehensive analysis, capturing even minimal phenolic content which might otherwise go undetected (Simčič, Stibilj, & Holcman, 2011). Next, the 20 uL of the sample was transferred into the 96 well plate in triplicate and 100 uL of Folin-Ciocalteu reagent with 80 uL of sodium carbonate were added into the sample well. In another well, the blank extract consisted of 20 uL of standard, 100 uL of distilled water and 80 uL of sodium carbonate. The blank extract also made in a triplicate manner. The blank diluent consisted of 20 uL DMSO and 100 uL of Folin-Ciocalteu reagent with 80 uL of sodium carbonate, Na_2CO_3. Lastly, the well plate was incubated for two (2) hours in the dark room before measuring the absorbance using a microplate reader at 750 nm. The TPC value was determined from the linear regression curve of absorbance against concentration using the equation $Y= mx + c$. Results obtained were expressed as microgram gallic acid equivalence per mg.

Total flavonoid content (TFC)

TFC for the standard gallic acid and the methanol crude were performed following the method by Herald et al. (2012) with minor modification.

TFC for standard catechin

The stock solution of catechin was prepared by two-

fold serial dilution in distilled water, resulting in eight different concentrations: 100, 50, 25, 12.5, 6.25, 3.125, 1.563, and 0.781 μg/mL. Each of these concentrations was then used to prepare the standards for the assay. Specifically, 25 μL of each concentration of the stock solution of catechin was transferred into a 96-well plate in triplicate. To each standard well, 100 μL of distilled water and 10 μL of sodium nitrate were added. The well plate was incubated for 5 minutes, and then 15 μL of aluminium chloride was added to the mixture. After an additional 6 minutes of incubation, 50 μL of sodium hydroxide and 50 μL of distilled water were added. In another set of wells, the blank consisted of 25 μL of the standard solution and 225 μL of distilled water. The blank was also prepared in triplicate. Lastly, the absorbance was measured using a microplate reader at 510 nm (Herald et al., 2012).

Estimation of TFC for sample extract

The 15 mg sample solution was prepared into two concentrations which were 5000 ug/ml and 10000 ug/ml. These concentrations were chosen to ensure robust detection and quantification of flavonoids within the plant extract. The selection of higher sample volumes is supported by research indicating that it enhances the sensitivity of the assay, allowing for accurate quantification of flavonoid content, even at lower concentrations (Ghasemzadeh et al., 2010). Flavonoids are typically present in higher concentrations compared to other phenolic compounds, and using larger sample volumes ensures that the assay can detect and quantify these compounds effectively. Next, the 25 uL of the sample was transferred into the 96 well plate in triplicate and 100 uL of distilled water with 10 uL of sodium nitrate was added into the standard well. The well plate was incubated for 5 minutes and then 15 uL of aluminium chloride was added to the mixture as well. After that, the well plate was incubated again for 6 minutes and 50 uL of sodium hydroxide together with 50 uL of distilled water was added. In another well, the blank diluent consisted of the same solution with extract replaced by DMSO solution. The blank diluent consisted of 25 uL of sample and 225 uL of distilled water. Lastly, the absorbance was measured using a microplate reader at 510 nm. The TFC value was determined from the linear regression curve of absorbance against concentration using the equation Y= mx + c. Results obtained were expressed as milligram of catechin equivalence per mg.

2,2-diphenyl-1-picrylhydrazyl Assay (DPPH Assay)

DPPH assay for the standard gallic acid and the methanol crude were performed following the method by Herald et al. (2012) with minor modification**.**

DPPH assay for standard ascorbic acid

The stock solution of 1 mg/mL ascorbic acid was prepared by two-fold serial dilution in distilled water, resulting in eight concentrations: 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 μg/mL. Each concentration was carefully pipetted with 50 μL into a 96-well plate in triplicate. Subsequently, 150 μL of DPPH solution was added to each well containing the standard ascorbic acid. The blank extract was prepared similarly, with the exception that 150 μL of methanol replaced the DPPH solution. This blank was also done in triplicate to account for any background absorbance. In another set of wells, a blank diluent was created by adding 150 μL of DPPH solution with 50 μL of DMSO. Additionally, a blank control was prepared using 50 μL of DMSO and 150 μL of methanol. Both blanks were included to ensure the accuracy of absorbance readings and were also done in triplicate. Following preparation, the 96-well plate was incubated for 40 minutes in a dark room to allow the reaction between ascorbic acid and DPPH to proceed. Absorbance was then measured at 515 nm using a microplate reader.

DPPH assay for sample extract

The 10 mg/mL sample extract was prepared by two-fold serial dilution in distilled water, resulting in eight concentrations: 10000, 5000, 2500, 1250, 625, 312.5, 156.25, and 78.125 μg/mL. Each concentration was pipetted with 50 μL into a 96-well plate in triplicate. Subsequently, 150 μL of DPPH solution was added to each well containing the sample extract. The blank extract was prepared similarly, with the exception that 150 μL of methanol replaced the DPPH solution. This blank was also done in triplicate to account for any background absorbance. In another set of wells, a blank diluent was created by adding 150 μL of DPPH solution with 50 μL of DMSO. Additionally, a blank control was prepared using 50 μL of DMSO and 150 μL of methanol. Both blanks were included to ensure the accuracy of absorbance readings and were also done in triplicate. Following preparation, the 96-well plate was incubated for 40 minutes in a dark room to allow the reaction between the sample extract and DPPH to proceed. Absorbance was then measured at 515 nm using a microplate reader. The decision to use a higher starting concentration of 10000 μg/mL

for the sample, compared to the standard ascorbic acid concentration of 1000 µg/mL, was based on the observed low DPPH values during preliminary testing. In order to ensure that the antioxidant activity of the samples could be reliably detected and quantified, a higher sample concentration was necessary. Due to the complex matrix of plant extracts and the variability in flavonoid content among different species, higher concentrations are often required to achieve measurable antioxidant effects. This approach is supported by the need to overcome any dilution effects and to enhance the sensitivity of the assay, ensuring that even subtle antioxidant activities can be detected and accurately quantified (Ghasemzadeh et al., 2010). Based on the absorbance result, the calculation was made using the formula (2) to get the percentage of inhibition and IC_{50}.

$$\frac{Percentage\ of\ inhibition\ (100\%)}{DPPH} = \frac{Control\ Absorbance\ -\ \text{Extract Absorbance}}{Control\ Absorbance} \times 100 \quad (2)$$

Results and discussion

Percentage yield

Mass of methanol crude was 7.40 g with the percentage yield of 4.95%, indicated a very poor extraction yield from the maceration process, falling below 20%. Maceration extraction involves soaking plant material in a solvent to extract bioactive compounds over time. This method is chosen for its simplicity, cost-effectiveness, and versatility in extracting a wide range of compounds without specialized equipment (Mbah & Eme, 2017). It is particularly effective for extracting phenolic compounds and flavonoids, which are known for their antioxidant properties and health benefits (Yazdani et al., 2021). This could have been caused by various factors. Some articles agreed that maceration was not an efficient method, as it typically resulted in low yields, was time-consuming, and caused cell degradation. Instead of using maceration, Soxhlet extraction was recommended because it could produce higher yields without degrading the bioactive compounds (Aspé & Fernández, 2011). Furthermore, some articles suggested that percolation was more efficient than maceration due to its continuous process, where the saturated solvent was constantly replaced by fresh solvent (Zhang et al., 2018). Regarding the maceration process itself, the low yield production might have occurred due to improper handling during the process, such as frequent transfers to another beaker that led to a loss of yield. Other than that, it might have also been caused by a lack of stirring and insufficient time during maceration because some research suggested macerating the sample for two weeks to provide better results (Aspé & Fernández, 2011).

Phytochemical screening test

From the result for phytochemical screening test (Table 1), the flavonoid and carotenoid tests were negative while steroid and terpenoid tests were positive. Phytochemical screening was a laboratory technique used to identify and analyse the presence of bioactive compounds in plant extracts. These compounds, known as phytochemicals, included various secondary metabolites such as alkaloids, flavonoids, terpenoids, and phenolic compounds. From the phytochemical screening result above, the steroid and terpenoid tests were positive, while the flavonoid and carotenoid tests were negative. Based on the studies conducted for the leaves part of *C.manillana*, the compound presence included flavonoid, terpenoid, carotenoid, and steroid (Y. Ragasa et al., 2015). The presence of terpenoid and steroid aligned with the studies conducted before for the leaf part. The negative result for flavonoid and carotenoid can be explained through two possible reasons. First, flavonoid and carotenoid compounds were absent in the stem bark of *C.manillana*, which is acceptable because none of the research or studies has been done for the stem bark, so no comparison can be made, and this can be a new finding in the herbal field. Secondly, the negative result may be caused by the low amount of flavonoid and carotenoid compounds present inside the sample, causing it to fall below the detection limit. Phytochemical screening tests are qualitative tests; hence, it is predictable that certain results may not be accurate (Hashmi & Bibi, 2021). Due to that, it is suggested to conduct further investigation through quantitative methods such as total flavonoid and phenolic content tests to obtain accurate and precise results.

Thin layer chromatography

From TLC test (Table 2), the iodine vapor, vanillin/ H_2SO_4 and UV light tests were positive while ferric chloride test was negative.

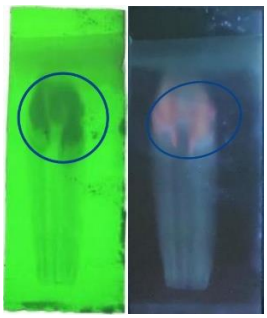
Table 1: The results of phytochemical screening test

Phytochemical Test	Observation
Steroid test	Result: Positive  The formation of a brown ring at the junction of two layers indicated the presence of steroids. The formation of a brown ring at the junction of two layers is indicative of the presence of steroids, as detected by the Liebermann-Burchard test in phytochemical analysis. This reaction involves the interaction of steroids with concentrated sulfuric acid and acetic anhydride, leading to the formation of a colored complex at the interface. Steroids undergo dehydration and subsequent rearrangement reactions in the acidic medium, resulting in the production of conjugated dienes which react with acetic anhydride to form the characteristic brown color (Zhang, Zheng, & Pan, 2015)
Carotenoid test	Result: Negative  No blue color formed, indicating that carotenoids could not be detected inside the sample.
Flavonoid test	Result: Negative  No yellowish color formed, indicating that flavonoids could not be detected in this test
Terpenoid test	Result: Positive  The formation of reddish-brown color shows the presence of terpenoids. Specifically, in the case of the Liebermann-Burchard test for terpenoids, the compounds undergo dehydration and subsequent rearrangement reactions in the acidic environment. This process leads to the formation of conjugated polyenes, which absorb light at specific wavelengths, resulting in the observed reddish-brown coloration at the reaction site (Trease & Evans, 2009)

Discussion of TLC

The mobile phase consisting of ethyl acetate and methanol in a ratio of 8:2 was chosen for TLC due to its effective separation capabilities for compounds with varying polarities. Ethyl acetate, being less polar, facilitates the elution of non-polar to moderately polar compounds, while methanol, with its higher polarity, aids in separating more polar compounds. This specific ratio is well-established in chromatography literature for providing clear spot development and good resolution of natural products, including terpenoids, flavonoids, and phenolic compounds (Ezzat et al., 2014). Therefore, ethyl acetate: methanol, 8:2 is ideal for analysing complex mixtures, such as those found in the stem bark of *C. manillana*, ensuring effective compound separation and identification on TLC plates. From the TLC results, all tests were positive

Table 2: The results of TLC tests

Phytochemical Test	Observation
Iodine vapour	Result: Positive  The presence of brown and yellow colors on the TLC plate after being sprayed with iodine vapor indicated the presence of compounds with double and triple bonds in the sample
Ferric chloride	Result: Negative The absence of dark blue spots after being sprayed with ferric chloride solution indicated that the phenolic compound was undetected using TLC. However, it is important to note that "undetected" does not necessarily mean absent; it could indicate that the amount present was too low to be detected by the TLC method (Wilson et al., 2014).
UV_{254} & UV_{366}	Result: Positive  The presence of black and fluorescent colors on the TLC plate under UV light indicated that the sample contained aromatic compounds.
Vanillin/ H_2SO_4	Result: Positive  The presence of many colors, such as pink, purple, and grey on the TLC plate after being sprayed with vanillin, shows that the sample contains terpenoid compounds.

except for the ferric chloride test. The iodine vapor test detects the presence of double bond compounds, indicated by the formation of a brown colour on the TLC plate. Aromatic compounds are detected using the UV test, appearing as black or fluorescent colours under UV light. The vanillin test is employed to detect terpenoid compounds, characterized by the formation of various colours such as grey, pink, purple, and blue. The ferric chloride test, typically used for detecting phenolic compounds, shows as blue spots on the TLC plate (Yahyaoui et al., 2017). TLC serves as a preliminary screening method to identify major compounds qualitatively (Wilson et al., 2014). However, due to the complex nature of the sample containing numerous active biochemicals, the spots on the TLC plate may not be clearly defined. This complexity indicates that the sample does not need to be

purified thoroughly for this study's purposes. The negative result from the ferric chloride test may not accurately reflect the presence of phenolic compounds, as their concentration might be too low to detect with this method. Hence, for a more precise determination of phenolic content, additional quantitative tests such as TPC and TFC are essential. Therefore, TLC serves as a general guidance in this study for screening major compounds, providing preliminary insights into the presence of double bond compounds, aromatic compounds, and terpenoids. However, for accurate quantitative analysis, TPC and TFC tests are necessary to determine the exact composition and concentration of phenolic and flavonoid compounds in the stem bark of *C. manillana*.

Total phenolic and flavonoid content test

Total phenolic content test

From the observation of Fig. 2: The 96 well plate of the sample for TPC, the blue colour solution formed indicated the presence of phenol compound. The intensity of the blue colour was very low which might be caused by the low amount of phenol presence inside the extract (Aryal et al., 2019).

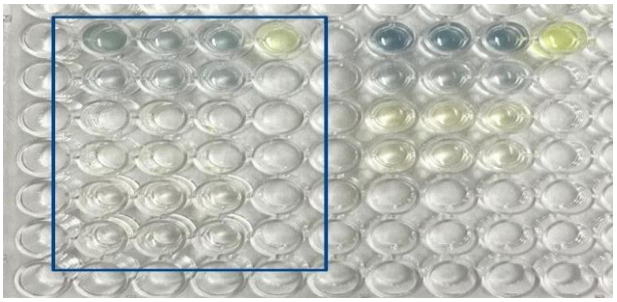
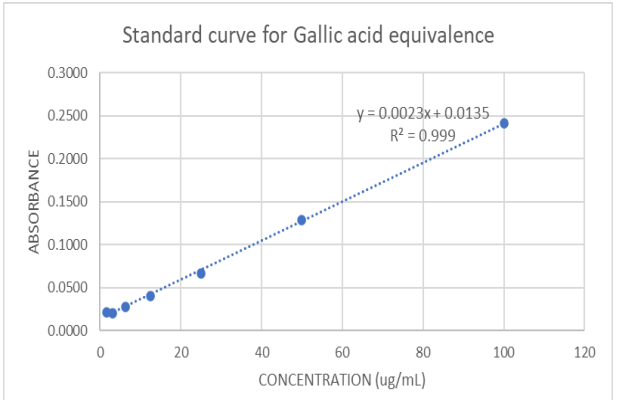


Fig. 2: The 96 well plate of the sample for TPC



Graph 1: The standard curve for Gallic acid equivalence

Based on the data interpretation by using the standard curve of gallic acid (Graph 1) the absorbance for TPC 10000 ug/mL (10 mg/mL) is 0.297 and Further calculation gave the value of TPC is 12.326 mg GAE/g. Further calculation of the mean TPC (mg GAE/g) for 10000 ug/mL is 11.630 ± 3.480 mg GAE/g and for 5000 ug/mL is 3.990 ± 0.260 mg GAE/g.

Total flavonoid content test

From the observation of **Error! Reference source not found.**, the yellow color solution formed indicated the presence of flavonoid compound. The intensity of the yellow color was very low which might be caused by the low amount of flavonoid presence inside the extract.

Based on the data interpretation by using the standard curve of catechin (Graph 2), the value of absorbance for TFC 10000 ug/mL (10 mg/mL) is 0.136. Further calculation gave the value of total flavonoid content is 0.995 mg CE/g. Further calculation of the mean TFC value for 10000 ug/mL is 0.937 ± 0.250 mg CE/g and for 5000 ug/mL is 0.428 ± 0.180 mg CE/g.

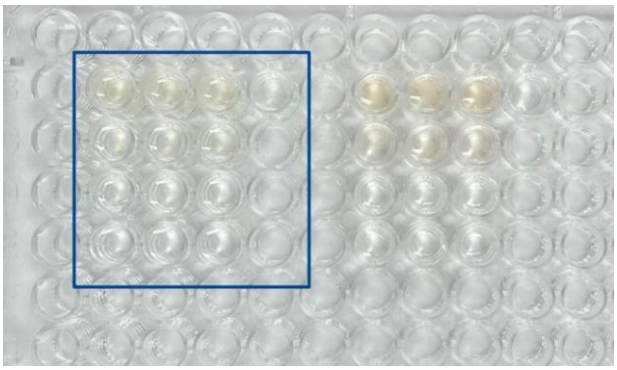
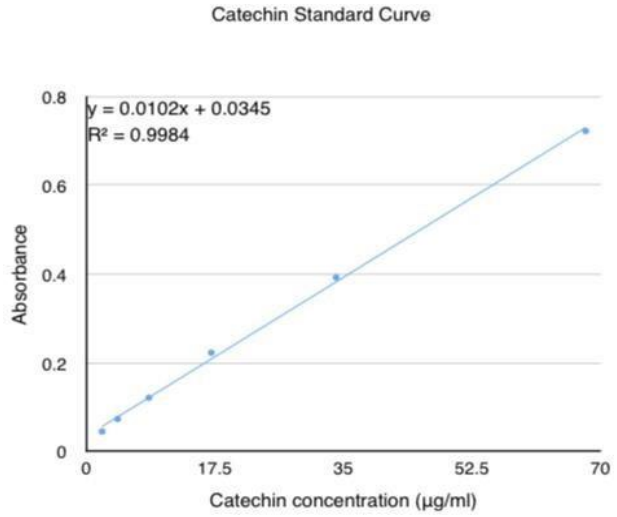


Fig. 3: The 96 well plate of the sample for TFC



Graph 2: The standard curve of catechin equivalent

Discussion

The TPC and TFC test was a quantitative method that provided an accurate measure of the phenolic

and flavonoid compounds present in a plant. This assay was significant in measuring the total antioxidant capacity, as high phenolic and high flavonoid content have been linked to high antioxidant capacity (Molole et al., 2022). In the case of *C. manillana* stem bark, the tests revealed a total phenolic content of 12.33 mg GAE/gram and 0.99 mg CE/gram for flavonoids. Since no prior studies on *C. manillana*'s stem bark existed for comparison, a reference value could not be established, making this a new finding in the herbal field. However, when comparing it with *Opilia amentacea*, a plant from the same family (Opiliaceae), its stem bark exhibited a significantly higher total phenolic content of 55.08 mg GAE/gram and a flavonoid content of 23.62 mg CE/gram (Ollo Youl et al., 2023). These large differences were expected since they belong to different species. One certainty from the TPC and TFC tests was that the presence of phenolic compounds and flavonoid compounds in the stem bark could be verified. Unlike the TLC and phytochemical test, which doesn't capable of detecting phenolic and flavonoids compound, the TPC and TFC tests quantitatively validated the existence of phenolic compounds and flavonoids. The negative results from the phytochemical tests and TLC do not conclusively indicate the absence of phenolic and flavonoid compounds. Instead, these results suggest that these compounds may be present but at levels below the detection limits of these qualitative methods. This further underscore the importance of employing quantitative tests such as TPC and TFC, which are capable of accurately detecting and quantifying even the lowest amounts of these bioactive compounds. Regarding colour changes for TPC, the reaction between the Folin-Ciocalteu reagent and phenolic compounds formed a blue-coloured solution. The blue color intensity was very low since the compound was also low in amount. For the TFC color changes, the yellow color formed from the formation of a complex between the aluminium ion and the carbonyl and hydroxyl groups of flavonoids. The mean TFC value was 0.937 ± 0.25 mg CE/g for 10000 µg/mL and 0.428 ± 0.18 mg CE/g for 5000 µg/mL. For TPC values, the mean for 10000 µg/mL was 11.630 ± 3.480 mg GAE/g and for 5000 µg/mL was 3.990 ± 0.260 mg GAE/g.

DPPH assay

Ascorbic acid

Ascorbic acid was used to serve as standard in this assay.

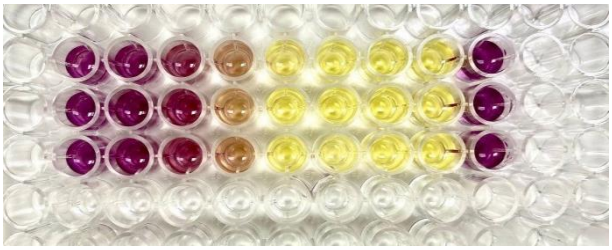


Fig. 4: The 96 well plate of the ascorbic acid for DPPH (from left; the lowest concentration to the highest concentration)

From the observation of Fig. 4, as the concentration of the sample increased, the purple color started to change to yellow, indicating the presence of antioxidant activity. The change in color occurred when the stable free radical compound in purple DPPH was scavenged, causing it to turn yellow. Based on the calculations, the IC_{50} for ascorbic acid is 6.730 µg/mL, and the mean IC_{50} is 7.100 ± 1.489 µg/mL.

Sample extract

From the observation of Fig. 5, the yellow colour indicates the presence of antioxidant activity. As the concentration of the sample reduced, the yellow colour has no longer seen, since the amount of antioxidant activity is too low scavenged the free radical of DPPH solution.

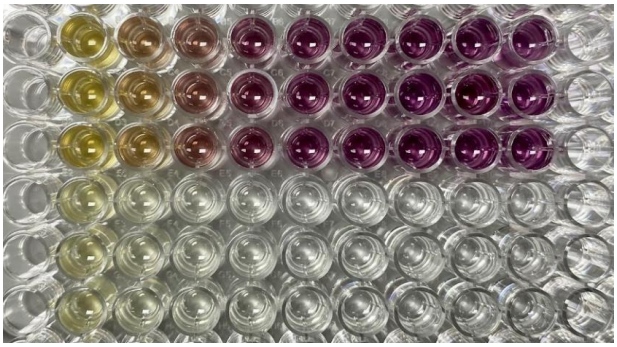
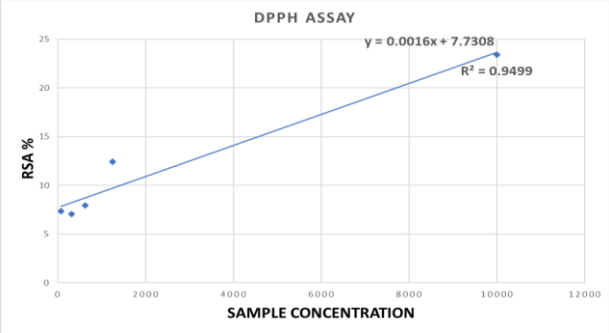


Fig. 5: The 96 well plate of the sample for DPPH (from left; the highest concentration to the lowest concentration)

The DPPH assay was conducted to measure the total antioxidant activity present in the plant extract. DPPH stands for 2,2-diphenyl-1-picrylhydrazyl, which exists in both solid and solution forms as a monomer. It is a stable free radical compound with a purple color at 517 nm that turns yellow when scavenged. The color change occurs due to the neutralization of DPPH when it receives an electron donated by an antioxidant compound, acting as an indicator of antioxidant activity (Munteanu & Apetrei, 2021). For this project, the graph was



Graph 3: The DPPH assay graph for sample

created using five concentrations instead of eight to achieve an R value above 0.95. As seen in Graph 3, the R value of 0.95 was achieved with concentrations of 10000, 5000, 2500, 1250, and 625 µg/mL.

The antioxidant activity by the DPPH neutralization method is often reported as IC_{50}, which is defined as the inhibitory concentration of the antioxidant necessary to scavenge 50% of the DPPH initial radical. The lower the IC_{50} value, the higher the potency of the antioxidant. From the data obtained from Graph 3, the IC_{50} calculated for the ascorbic acid was 6.730 ug/mL, while the IC_{50} calculated for *C. manillana* stem bark was 26 mg/mL. This showed that the antioxidant value in ascorbic acid was higher than the one present inside *C. manillana* stem bark. Besides, it can be said that the antioxidant properties of stem bark were very weak since it was above 0.1 mg/mL (Welz et al., 2018). Hence, it can be concluded that the stem bark of *C. manillana* is not a good source of antioxidants. The highest percentage inhibition was 23.4% at 10000 ug/mL. None of the studies have been made for the stem bark, so this might be a new finding available. Comparing to the studies done for the leaves part, the percentage inhibition was higher with 31% compared to the stem bark. The low antioxidant activity may be associated with the low amount of flavonoid and phenolic compound since they are considered as natural potent antioxidants (Tungmunnithum et al., 2018).

Conclusion

From the tests that have been conducted, the phytochemical screening showed a positive result for the presence of steroids and terpenoids, while yielding negative results for flavonoids and carotenoids. In the TLC test, the iodine vapor test, vanillin test, and UV light test were positive, indicating the presence of double bond compounds, terpenoids, and aromatic compounds respectively. However, the ferric chloride test was negative, suggesting the absence of phenolic compounds. Despite this, both TLC and phytochemical screening tests are qualitative methods, and their outcomes may be limited in accuracy. Next, the TFC and TPC result shows the presence of flavonoid with 0.995 mg CAE/g and phenolic with 12.326 mg GAE/g. The antioxidant activity for *C. manillana* stem bark was low, with an IC_{50} at 26 mg/mL (>0.1 mg/mL), and the highest percentage inhibition is 23.4% at 10,000 µg/mL. For future studies, multiple extractions should be performed to extract and quantify targeted compounds that provide antioxidant activity, such as flavonoids, phenolic compounds, and terpenoids. The maceration process can also be replaced with a Soxhlet or percolation process. Lastly, instead of using methanol as the solvent, ethanol can be considered for better safety reasons. In conclusion, this study can provide valuable information for future research and may serve as a basis for authorities to consider preserving this plant due to its beneficial value.

Authors contributions

Study design, A.S.A. Direction and Coordination, S.Z.M.S. Investigation, A.S.A. Resources, S.Z.M.S. Writing-Original Draft, A.S.A Writing-Review and Editing S.Z.M.S., and A.S.A. Supervision, S.Z.M.S. Project Administration, S.Z.M.S. and A.S.A.

Conflict of interest

The authors claim that there is no conflict of interest associated with this work.

References

Ajayi, & Ajibade O. (2011). Preliminary Phytochemical Analysis of some Plant Seeds. *J.Chem.Sci. Research Journal of Chemical Sciences*, *1*(3). Retrieved from https://www.isca.me/rjcs/Archives/v1/i3/08.pdf

Ameerah Shaeroun, A. R., Hamed Alqamoudy, A. B. A., Mohamed, Khalifa. S., Nouri Kushlaf, N. A., Akram Almabrouk misbah, A. M. EL-mahmoudy., & Zuhur rajab Almes, S. T. O. (2019). Thin Layer Chromatography (TLC) and Phytochemical Analysis of *Moringa Oleifera* Methanol, Ethanol, Water and Ethyl Acetate Extracts. *Saudi Journal of Medical and Pharmaceutical Sciences*, *05*(10),

817–820. https://doi.org/10.36348/sjmps.2019.v05i10.002

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic Content, Flavonoid Content and Antioxidant Potential of Wild Vegetables from Western Nepal. *Plants*, *8*(4), 96. https://doi.org/10.3390/plants8040096

Aspé, E., & Fernández, K. (2011). The effect of different extraction techniques on extraction yield, total phenolic, and anti-radical capacity of extracts from *Pinus radiata* Bark. *Industrial Crops and Products*, *34*(1), 838–844. https://doi.org/10.1016/j.indcrop.2011.02.002

Bogacz-Radomska, L., & Harasym, J. (2018). β-Carotene—properties and production methods. *Food Quality and Safety*, *2*(2), 69–74. https://doi.org/10.1093/fqsafe/fyy004

Carvalho, A. M. S., Heimfarth, L., Pereira, E. W. M., Oliveira, F. S., Menezes, I. R. A., Coutinho, H. D. M., … Quintans-Júnior, L. J. (2020). Phytol, a Chlorophyll Component, Produces Antihyperalgesic, Anti-inflammatory, and Antiarthritic Effects: Possible NFκB Pathway Involvement and Reduced Levels of the Proinflammatory Cytokines TNF-α and IL-6. *Journal of Natural Products*, *83*(4), 1107–1117. https://doi.org/10.1021/acs.jnatprod.9b01116

Cempereia manillana Merr. – Opiliaceae – 81 – Forest of 'Ilm. (2022, July 19). Retrieved June 16, 2024, from Forest of Ilm KOP IIUM website: https://forest-ilm.iium.edu.my/81-cempereia-manillana/

Denny, Marfuah Wardani & Adi Susilo. (2021). Diversity and potential utilization of medicinal plants in Way Kambas National Park. *IOP Conference Series*, *914*(1), 012001–012001. https://doi.org/10.1088/1755-1315/914/1/012001

Deshmukh, M. A., & Theng, M. A. (2018). Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolite of *Acacia arabica* Bark. *International Journal of Current Pharmaceutical Research*, *10*(2), 35. https://doi.org/10.22159/ijcpr.2018v10i2.25889

Dubale, S., Kebebe, D., Zeynudin, A., Abdissa, N., & Suleman, S. (2023). Phytochemical Screening and Antimicrobial Activity Evaluation of Selected Medicinal Plants in Ethiopia. *Journal of Experimental Pharmacology*, *Volume 15*, 51–62. https://doi.org/10.2147/jep.s379805.

Ezzat, S. M., Salama, M. M., Seif el-Din, S. H., & Saleh, S. (2014). Chemotaxonomic and biological studies of the genus Euphorbia in Egypt. Turkish Journal of Botany, 38(3), 485-500.

Goltz, S. R., Campbell, W. W., Chitchumroonchokchai, C., Failla, M. L., & Ferruzzi, M. G. (2012). Meal triacylglycerol profile modulates postprandial absorption of carotenoids in humans. *Molecular Nutrition & Food Research, 56*(6), 866-877

Hashmi, H. F., & Bibi, S. (2021). Qualitative and Quantitative Analysis of Phytochemicals in *Lepidium pinnatifidum* Ledeb. *Scholars International Journal of Traditional and Complementary Medicine*. https://doi.org/10.36348/sijtcm.2021.v04i05.002

Herald, T. J., Gadgil, P., & Tilley, M. (2012). High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agriculture*, *92*(11), 2326–2331. https://doi.org/10.1002/jsfa.5633

K. Jeyaprakash, & N. Balachandran. (2018). *Champereia manillana (Blume) Merr. (Opiliaceae) and Stemodiaverticillata (Mill.) Hassl. (Plantaginaceae): New distributional records to North East India.*

Lawag, I. L., Nolden, E. S., Schaper, A. A. M., Lim, L. Y., & Locher, C. (2023). A Modified Folin-Ciocalteu Assay for the Determination of Total Phenolics Content in Honey. *Applied Sciences*, *13*(4), 2135. https://doi.org/10.3390/app13042135

Mbah, C. J., & Eme, P. E. (2017). Comparative study of the effects of different extraction techniques on antioxidant properties of *Ficus capensis*. *International Journal of Plant Research, 7*(1), 1-8.

Micera, M., Botto, A., Geddo, F., Antoniotti, S., Bertea, C. M., Levi, R., … Querio, G. (2020). Squalene: More than a Step toward Sterols. *Antioxidants*, *9*(8), 688. https://doi.org/10.3390/antiox9080688

Munteanu, I. G., & Apetrei, C. (2021). Analytical Methods Used in Determining Antioxidant Activity: A Review. *International Journal of Molecular Sciences*, *22*(7), 3380. https://doi.org/10.3390/ijms22073380

Musa, H. H., Musa, T. H., Oderinde, O., Musa, I. H., Shonekan, O. O., Akintunde, T. Y., & Onasanya, A. K. (2022). Traditional herbal medicine: overview of research indexed in the scopus database. *Advances in Traditional Medicine*. https://doi.org/10.1007/s13596-022-00670-2

Molole, G. J., Gure, A., & Abdissa, N. (2022). Determination of total phenolic content and antioxidant activity of *Commiphora mollis* (Oliv.) Engl. resin. *BMC Chemistry*, *16*(1). https://doi.org/10.1186/s13065-022-00841-x

N.T.R., M. (2013a). Antioxidant activity of phenolic and flavonoid fractions of *Cleome gynandra* and *Maerua angolensis* of Burkina Faso. *Journal of Applied Pharmaceutical Science*. https://doi.org/10.7324/japs.2013.30207

N.T.R., M. (2013b). Antioxidant activity of phenolic and flavonoid fractions of Cleome gynandra and Maerua angolensis of Burkina Faso. *Journal of Applied Pharmaceutical Science*. https://doi.org/10.7324/japs.2013.30207

Ollo Youl, Ramata, B., Sibidou Yougbaré, Boubacar Yaro, Tata Kadiatou Traoré, Raïnatou Boly, Josias B. Gérard Yaméogo, Moumouni Koala, Noufou Ouédraogo, Elie Kabré, Tinto, H., Maminata Traoré-Coulibaly, & Adama Hilou. (2023). Phytochemical Screening, Polyphenol and Flavonoid Contents, and Antioxidant and Antimicrobial Activities of *Opilia amentacea* Roxb. (Opiliaceae) Extracts. *Applied Biosciences*, *2*(3), 493–512. https://doi.org/10.3390/applbiosci2030031

Qin, Z., Liu, H.-M., Ma, Y.-X., & Wang, X.-D. (2021). Developments in extraction, purification, and structural elucidation of proanthocyanidins (2000–2019). *Bioactive Natural Products*, 347–391. https://doi.org/10.1016/b978-0-12- 819485-0.00008-6

Rahman, A., Rindam Latief, & H Kartono. (2023). Extraction and analysis of lutein and antioxidant activities from red spinach's root, stem, and leaf. IOP Conference Series, 1200(1), 012021–012021. https://doi.org/10.1088/1755-1315/1200/1/012021

Simčič, M., Stibilj, V., & Holcman, A. (2011). Fatty acid composition of eggs produced by the *Slovenian autochthonous* Styrian hen. *Food Chemistry*, *125*(3), 873–877. https://doi.org/10.1016/j.foodchem.2010.09.055

Trease, G. E., & Evans, W. C. (2009). *Pharmacognosy* (16th ed.). SaundersElsevier.

Tungmunnithum, Thongboonyou, A., Pholboon, A. ,&Yangsabai, A. (2018). Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: An Overview. *Medicines*, *5*(3), 93. https://doi.org/10.3390/medicines5030093

Welz, A. N., Emberger-Klein, A., & Menrad, K. (2018). Why people use herbal medicine: insights from a focus-group study in Germany. *BMC Complementary and Alternative Medicine*, *18*(92). https://doi.org/10.1186/s12906-018-2160-6

Wilson, C. R., Butz, J. K., & Mengel, M. C. (2014). Methods for Analysis of Gastrointestinal Toxicants☆. Reference Module in Biomedical Sciences. https://doi.org/10.1016/b978-0-12-801238-3.02125-5

Yahyaoui, O. E., Ouaaziz, N. A., Guinda, I., Sammama, A., Kerrouri, S., Bouabid, B., Bakkall, M. E., Quyou, A., Lrhorfi, L. A., & Bengueddour, R. (2017). Phytochemical screening and thin layer chromatography of two medicinal plants: *Adansonia digitata* (Bombacaceae) and *Acacia raddiana* (Fabaceae). *Journal of Pharmacognosy and Phytochemistry*, 6(1):10-15.

Yang, G.-S., Wang, Y.-H., Wang, Y.-H., & Shen, S.-K. (2017). The complete chloroplast genome of a vulnerable species *Champereia manillana* (Opiliaceae). *Conservation Genetics Resources*, *9*(3), 415–418. https://doi.org/10.1007/s12686-017-0697-1

Yazdani, M., Hojjati, M., Sharifani, M., & Shariati, M. A. (2021). Phytochemical analysis and antioxidant activity of some selected medicinal plants. *Journal of Applied Research on Medicinal and Aromatic Plants, 21*, Article 100307

Y. Ragasa, C., A. Ulep, R., Vincent Antonio S. Ng, & Robert Brkljača. (2015). Chemical Constituents of *Champereia manillana* (Blume) Merrill. *Scholar Research Library*, 1–6. Retrieved from https://www.scholarsresearchlibrary.com/articles/chemical-constituents-of-champereia-manillana-blume-merrill.pdf

Zhang, Q.-W., Lin, L.-G., & Ye, W.-C. (2018). Techniques for Extraction and Isolation of Natural products: a Comprehensive Review. *Chinese Medicine*, *13*(1). https://doi.org/10.1186/s13020-018-0177-

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Preparation, characterization, and bioactivity evaluation of curcumin-loaded poly (lactic-co-glycolic acid) nanoparticles

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Abstract

Background: One of the main challenges with curcumin is its hydrophobic nature, which limits its solubility and bioavailability. This issue can be addressed by using poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs). The small size and large surface area of these NPs significantly enhance drug delivery systems by improving the solubility and bioavailability of the drug. **Objective**: This project focuses on the preparation, characterization, and bioactivity evaluation of curcumin loaded in PLGA NPs, intended for the delivery of curcumin extracted from *Curcuma xanthorrhiza*, commonly known as 'temulawak' or 'Java turmeric'. **Methodology**: Curcumin was extracted and stored at 4°C for testing. PLGA-curcumin NPs were synthesized using the single emulsion method. Nanoparticle morphology was analyzed using SEM, while particle size and zeta potential were measured with a Zetasizer. Entrapment efficiency and drug loading capacity were calculated. *In vitro* release studies in phosphate buffer were conducted using UV-visible spectrophotometry. The cytotoxicity of the curcumin-loaded NPs was tested on MCF-7 breast cancer cells using the MTT assay. Statistical analyses were performed using Minitab 14, and Microsoft Excel was used for graphical representations, with significance set at $p < 0.05$. **Results**: The mean particle size of the curcumin NPs was 498.9 nm ± 597.4 nm. The entrapment efficiency and drug loading capacity were 50% and 5%, respectively. The average zeta potential was recorded as -28.7 mV ± 6.19 mV. The *in vitro* release study did not produce significant results as low concentrations of curcumin were detected. However, the bioactivity of the curcumin-loaded PLGA NPs demonstrated lower cell viability compared to the curcumin extract, suggesting that the PLGA formulation is more effective at inducing cancer cell death. This indicates its potential as a more efficient therapeutic option in cancer treatment. **Conclusion**: The single emulsion method managed to produce nano-sized particles with good zeta potential and bioactivity on MCF-7 cells. However, further study needs to be done to produce better formulation which can increase entrapment efficiency, drug loading capacity and also *in vitro* release profile.

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Introduction

Nowadays, NPs have become one of the popular terms used in drug delivery systems. NPs are particulate materials with at least one dimension less than 100 nm (Khan et al., 2019). The small size and large surface area of NPs have exhibited great advantages in drug delivery systems such as increasing solubility of the drug which will increase the bioavailability of the drug. Many drugs show problems in their solubility which now can be counteracted with the technology of NPs. By using NPs, more specific drug-targeted activity can be achieved which will increase the therapeutic value of a drug for the consumers. Nano-curcumin has been found to be an ideal choice in drug delivery systems compared to free curcumin. Organs that curcumin can hardly reach can be accessed by nano curcumin (Karthikeyan et al., 2020). It was discovered that nano curcumin may have a greater capability for intracellular absorption than regular curcumin. Targeting intracellular microorganisms for infectious diseases requires this property as well. When compared to free curcumin, it has been found that nano curcumin has a higher level of systemic bioavailability in the plasma and tissues. When compared to the therapy of native curcumin in an investigation with rat models, nano curcumin delivers a 60-fold increase in the biological half-life, increasing the in vivo bioavailability and distribution of the tissues (Karthikeyan et al., 2020).

One commonly used copolymer in drug delivery systems is poly (lactic-co-glycolic acid) (PLGA). It possesses excellent biodegradability and biocompatibility, which has enabled it to gain approval from the Food and Drug Administration. Based on its name, PLGA is made from the copolymer of polyglycolic acid (PGA) and polylactic acid (PLA). In the field of production of bone substitute structures, the co-polymer PLGA is typically favored over its component homopolymers PLA and PGA because it provides better control over degrading qualities by adjusting the ratio between its monomers. The degree of crystallinity is decreased when the PLA is copolymerized with the crystalline PGA, which results in faster rates of hydration and hydrolysis (Lanao et al., 2013). The PLGA has also been used in the treatment of cancer, the healing of wounds, and for antibacterial, antioxidant, and anti-inflammatory purposes (Guo et al., 2023). The ratio between the two monomers will produce different types of PLGA. The most common type used is the ratio of 50:50. Since it is more hydrophobic than glycolic acid, lactic acid is often the monomer that is most numerous in PLGA. Lactic acid-rich PLGA polymers have slower rates of degradation and drug release. The copolymer with a 50:50 ratio is an outlier since it has the fastest breakdown rates and the shortest half-life (Lanao et al., 2013). The manufacture of PLGA particles involves several methods, such as single and double emulsion solvent evaporation (ESE), nanoprecipitation, spray-drying, microfluidics, and hydrogel templating (Garms et al., 2021). Bottom-up and top-down methods can be used to produce the NPs. When using top-down techniques, including emulsion solvent evaporation (single- or double-phase emulsions), pre-synthesized polymer chains, like PLGA, are used to physically produce the NPs (Operti et al., 2021). Meanwhile, PLGA NPs can be chemically synthesized using lactide and glycolide monomers through bottom-up techniques.

One of the favorable methods that can be used is the top-down method of single and double-emulsion solvent evaporation due to its simplicity and efficiency. Simply put, a polymer dissolves the medication or emulsifies it with a polymer in an organic phase before the aqueous phase is added. Particles are cleaned and collected using centrifugation after the solvent has evaporated in preparation for lyophilization and long-term storage (McCall & Sirianni, 2013). The benefit of this method is the flexibility to regulate the release kinetics of the substance that is encapsulated as well as particle size and polydispersity (Garms et al., 2021). The NPs are obtained by ultracentrifugation after the suspension is kept under stirring to allow the organic solvent to evaporate. This method provides for easy scaling up and enables particle size management.

Curcuma xanthorrhiza also known as *temulawak*, Java ginger, Javanese ginger, or Javanese turmeric

has been used traditionally in jamu (traditional medicine from Indonesia). According to data from 2019, *C. xanthorrhiza* was grown in Indonesia on a grand scale, with yields of 29,637,119 kg produced on a demolished harvested area of more than 13,042,873 m^2 (Rahmat et al., 2021). Javanese turmeric is known to possess various pharmacological activities such as antibacterial, antifungal, antioxidant, antihypertensive, antihepatotoxic, and antidiuretic properties (Salleh et al., 2016). This has become one of the factors which contribute to the usage of Javanese turmeric in jamu to treat certain diseases such as liver disease, anticancer, arthritis, and hypertension. The mRNA synthesis of pro-inflammatory mediators, including cytokines and related enzymes like cyclooxygenase (COX)-2 and inducible nitric oxide synthase (iNOS), is reduced by curcumin (Hassanzadeh et al., 2020). Terpenoids and curcuminoids were shown to be the most prevalent essential phytochemicals in the *C. xanthorrhiza* rhizome, according to scientific investigations (Rahmat et al., 2021). Curcuminoid is a polyphenol substance which produces the yellow color of turmeric. The activity of curcumin also emerges from curcuminoid. From the rhizome of *C. xanthorrhiza*, several curcuminoids, including octahydrocurcumin, 1- hydroxy-1,7-bis(4-hydroxy-3-methoxypheny1)-6-heptene-3,5-dione, dihydrocurcumin, hexa-hydrocurcumin, curcumin, monodemethoxy curcumin, bis-demethoxy curcumin, 1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4- hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,4-dione, 3-demethoxycyclocurcumin, and 1,7-bis(4-hydroxy-3- methoxyphenyl)-heptane-3,5-diol has been successfully extracted and classified (Rahmat et al., 2021). Curcuminoids make up about 2%–6% (w/w) of turmeric. In the latter, there is 2% bisdemethoxycurcumin, 18% demethoxycurcumin, and 80% curcumin (Gupta et al., 2013). The active substance of curcumin which can be found in Javanese turmeric possesses one problem which is hydrophobicity. One of the main problems with turmeric or curcumin is it possesses a hydrophobic characteristic which makes it possess low solubility which will reduce the availability of curcumin in drug delivery systems (Dei Cas & Ghidoni, 2019; Karthikeyan et al., 2020). Curcumin has a log P of 3.2, thus it is nearly insoluble in water. Curcumin possesses 30nM water solubility (Hegde et al., 2023). The aim of this experiment is to develop and evaluate curcumin-loaded PLGA NPs to enhance the solubility, bioavailability, and therapeutic efficacy of curcumin extracted from *Curcuma xanthorrhiza*.

Materials and methods

Curcuma xanthorrhiza (obtained from Mega Mendung, West Java, Indonesia) in September 2023, PLGA Lactide: Glycolide (75:25, MW: 66000-107,000) (Sigma-Aldrich), Dichloromethane, PVA (1% w/v) (Sigma-Aldrich), deionized water, sonicator (Qsonica), centrifuge (Supra 22K), analytical balance, spatula, filter paper, stainless steel grinder (XINGANBANGLE 3000W), rotary evaporator (BÜCHI Rotavapor R-205), Zetasizer, UV-vis spectrophotometer, MTT [3-(4, 5-dimethythiazol-2-yl)- 2,5- diphenyl tetrazolium bromide] assay, phosphate buffer saline, Dulbecco's Modification of Eagle's Medium, TrypLE Express (Gibco), 75 cm^2 tissue culture flask (Avantor), 25 cm^2 tissue culture flask (SDL), Tamoxifen (Sigma-Aldrich).

Extraction of Curcuminoids

The extraction of curcuminoids was done using the methodology of maceration with the aid of stirring. 58.6 g of turmeric rhizome powder sample was used and put into the beaker and 150 ml of ethanol 95% was added and the curcumin within the turmeric rhizome powder was extracted into the ethanol. This process was left for 1 day. The next day, the mixture was filtered using filter paper, and the dark brown extract was collected. The remaining powder was extracted two more times using the same solvent with the aid of a magnetic stirrer and left to stir for 2 hours. The collected extraction was cooled, condensed separately under reduced pressure using a rotary evaporator, and kept at 4°C prior to further testing.

Preparation of Curcumin Loaded PLGA

The PLGA-curcumin NPs were prepared using the single emulsion method retrieved from Arzani et al. (2018) with slight modification.100 mg of PLGA lactide: glycolide (75:25, MW: 66000-107,000) was dissolved within 10 ml of dichloromethane. 10 mg of C. xanthorriza extract was added to the PLGA-DCM mixture and allowed to dissolve completely. 100 ml of PVA (1% w/v) in water was introduced with a PLGA-curcumin mixture drop by drop while intermittent vortexing at a high setting. The mixture was sonicated at 40 amplitude for three minutes to form a fine emulsion after the PLGA-curcumin had been fully incorporated. The resulting dispersion of NPs was rotated on a magnetic stirrer at 800 rpm for 4 hours. The NPs were gathered by centrifugation for 25 minutes at 15000 rpm and washed 3 times with deionized water. The NPs were freeze-dried. The produced NPs were kept in storage until further use.

Evaluation and characterization of curcumin-loaded PLGA and bioactivity.

Morphology study using scanning electron microscope

The morphology of PLGA-curcumin NPs was observed using an SEM (Carl Zeiss AG - EVO®50) for particle visualization. Greater beam intensities may cause the sample to heat up locally, changing the particle's surface shape. At a magnification of 200X, microparticles may be viewed, and at 3,000X, they can be distinguished. The sample was coated with gold using an ion sputter after it had dried to the point of coating. This method was retrieved from McCall & Sirianni, (2013).

Nanoparticle size and zeta potential measurement

The size and polydispersity index of the nano-formulated curcumin particles were determined using a Zetasizer. The prepared NPs (1 mg/50 ml) at 40 W and 4°C for 10 minutes. Every measurement was made three times, and the average of the measurement was taken.

Determination of Entrapment Efficiency and Drug Loading Capacity

10 mg of curcumin NPs were diluted in 10 milliliters of distilled water and put into centrifugation tubes, which were then filled with 30 milliliters of water and centrifuged for thirty minutes at a speed of 10,000 rpm. This method is retrieved from Arozal et al., (2022). The EE and DLC were computed after obtaining the sample weights. EE calculation:

$$\text{Entrapment efficiency (EE)} = \frac{(C0-C1)}{C0} \boldsymbol{x}\ \mathbf{100}\% \qquad (1)$$

C0 = the weight of the active compound at first (mg)

C1 = free active compound weight (mg)

Calculation of DLC:

$$\text{Drug Loading Capacity: } \frac{(C0-C1)}{Ctotal} \boldsymbol{x}\ \mathbf{100}\% \qquad (2)$$

C0 = weight of active compound at first (mg)

C1 = free active compound weight (mg)

Ctotal = total weight of NPs (mg)

The yield of curcumin NPs was also calculated using the formula:

$$\text{Yield (\%)} = \frac{(m\ drug + m\ polymer)}{(m\ nanoparticles)} \boldsymbol{x}\ \mathbf{100}\% \qquad (3)$$

In vitro release of curcumin

A 500 mL container is filled with a quantity of the dissolution medium, which is 0.1 M phosphate buffer at 25℃ and pH 6.8. The dialysis membrane which contained 5 ml of curcumin NPs was inserted into the dissolution tester with a rotation of 50 rpm. The stopwatch was set in motion simultaneously, and the 3 ml of samples were taken at intervals of 15, 30, 45, 60, 90, 120, 150, 180, and 360 minutes. 3 mL of samples were taken from the area in the middle of the container, at least 1 cm from the wall's surface, and replaced by the same amount of fresh PBS. Every sample taken from the above time points was measured using a 1900-UV UV-visible spectrophotometer (Shimadzu, Japan) set to 423 nm.

The derived linear regression equation was then used to compute the concentration. Curcumin concentrations were also determined based on the amount of curcumin that was liberated from the matrix during testing. This method is retrieved from Arozal et al., 2020.

Cytotoxicity assay

The cytotoxicity study was carried out on breast cancer cells (MCF-7). The viability of the cells (3 × 105/well) was examined using the MTT [3-(4, 5-dimethythiazol-2-yl)- 2,5- diphenyl tetrazolium bromide] assay. This method is retrieved from Gnanamangai et al., (2019). The cells were sown in 96-well plates and cultured in a CO_2 incubator overnight. Following the application of curcumin extract and curcumin-loaded PLGA at a variety of concentrations, the cells are rinsed with phosphate buffer saline (PBS). Untreated cells are regarded as negative control and the cells treated with Tamoxifen (Sigma Aldrich) are considered as positive control. Then, each well received 20µl of MTT (5 mg/mL), and the wells were incubated at 37 °C for 3 to 4 hours. 100 µl of dimethyl sulfoxide (DMSO) was used to dissolve the purple and blue MTT formazan precipitate. The absorbance was measured using the ELISA reader at 570 nm wavelength. The following formula was used to determine the triplicate values:

$$\% \text{ of cell viability} = (\text{OD of treated cells}/\text{ OD of control cells}) \times 100 \quad (4)$$

Statistical Analysis

The mean ± SD was used to present the data. P-values below 0.05 were regarded as significant. Statistical analyses were performed using Minitab 14. Microsoft Excel was used to create graphical representations.

Results and discussion

Evaluation and characterization of curcumin-loaded PLGA and bioactivity.

Morphology study using scanning electron microscope.

The SEM examination results are shown in Figure 1 which is 1a: the NPs at 1000X, 1b: the NPs at 2000X magnification, 1c: the NPs at 3000X magnification and 1d: the NPs at 3000X magnification. The SEM image of these curcumin-loaded PLGA shows a round and spherical shape.

Because emulsion-based production processes are easier to process and optimize, they are preferred for the formation of polylactic acid NPs (PLGA NPs) for drug delivery applications (Garms et al., 2021). With the usage of a single emulsion method, the particles within the range of nanometers managed to be obtained. The submicron-sized particles function as carriers that mediate the drug model so that the drug goes straight to the destination in drug delivery models with controlled-release targets (Arozal et al., 2020). However, some particles are micrometers in size. This can be due to the formulation process. The molecular weight of PLGA used in this experiment is big which is 66000-107,000 Da. This produced polydispersity in the size of the particles produced. Another factor that can affect the production of microparticles is the possibility of an error in the single emulsion method, leading to non-uniform particle sizes. In addition, a short sonication time may increase the polydispersity of the particles. According to Halayqa & Domańska (2014), increasing the concentration of PLGA leads to larger NPs for both drugs, likely due to the higher viscosity of the organic phase. This increased viscosity reduces shear stress, resulting in larger droplets during emulsification. Moreover, higher viscosity reduces the dispersion of the organic phase into the aqueous phase, further contributing to larger nanoparticle size. Increasing the drug amount in the organic phase results in larger NPs, and higher sonication power significantly affects droplet size, leading to larger NPs and increased polydispersity (Halayqa & Domańska, 2014). By increasing the sonication power, temperature increases promoting droplet coalescence and subsequent nanoparticle size increase. From the research, it can be hypothesized that by reducing the concentration of PLGA, reducing amounts of drug in organic phase and low sonication power can reduce the size of the NPs.

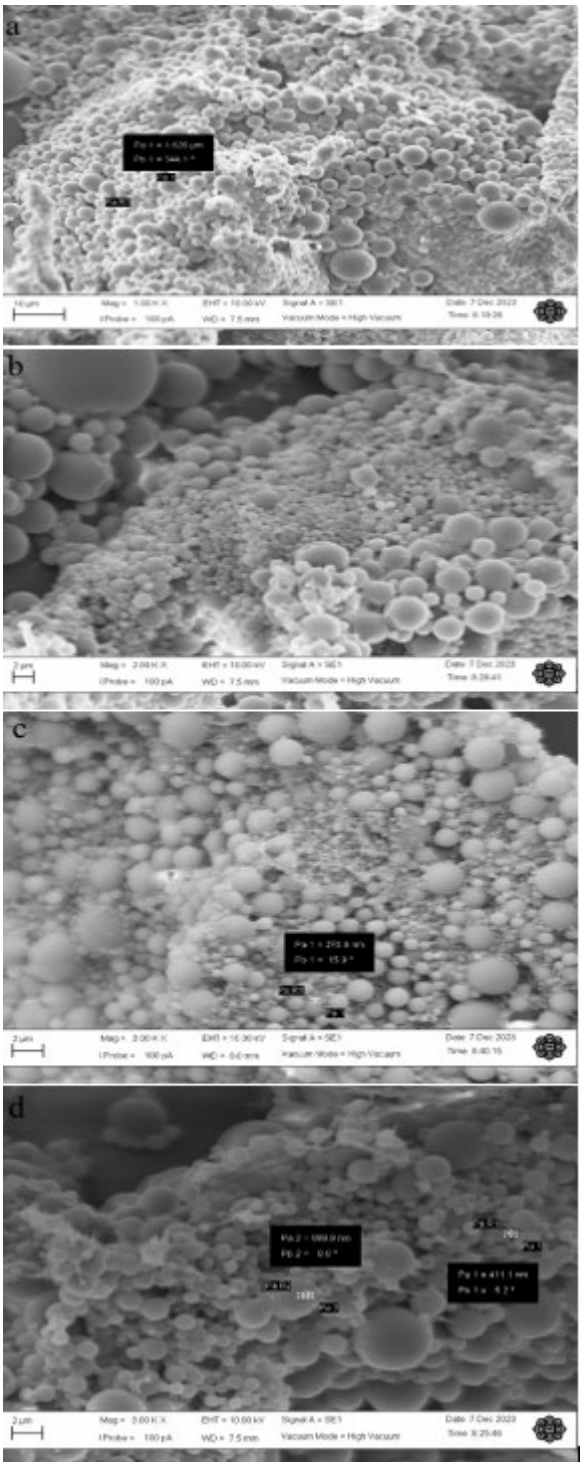

Fig. 1: Scanning electron microscopic images of curcumin-loaded PLGA NPs. 1(a) shows the NPs at 1000X. At this magnification, microparticle-sized PLGA can be seen with a size of 1.626 µm. At 2000X magnification (1b), smaller size molecules can be seen alongside big molecules (micro molecules). At 3000X magnification (1c and 1d), nanosized particles become more apparent with sizes ranging from 270.9 nm to 669.9 nm, respectively.

It is also stated in research done by Garms et al., (2021) that when comparing samples generated at a given concentration, SEM showed greater NP aggregation than TEM did. As a result, particles smaller than 50 nm were not visible in the SEM images, although 30 nm-sized particles were visible in the TEM images. Many formulation variables can be varied using the single emulsion approach, and these variations can change the characteristics of the NPs. For instance, bigger NPs with a wider size distribution will often be produced when dichloromethane (DCM) is used as the solvent instead. Because ethyl acetate (EtAc) is miscible with water, the polymer droplet in the main emulsion has less surface tension, which results in smaller NPs (McCall & Sirianni, 2013).

Curcumin has very poor solubility in water. In this experiment, PLGA and curcumin were diluted using dichloromethane. After the curcumin was dissolved into PLGA, the mixture was then dropped one drop at a time in distilled water where PVA had been dissolved.

The SEM examination shows that the NPs are round and sphere-shaped. The spherical shape indicates well-formed curcumin NPs that are more likely to pass across membranes. Round NPs are the most appropriate shape for drug delivery applications out of all of them (Arozal et al., 2020). From the usage of ImageJ, an image (Figure 1a) has been chosen to analyze how many NPs and microparticles are present. Based on the histogram in Figure 2a, it stated 315 particles of 356 particles are less than 1000 nanometers (1 micrometer) in diameter. This represents 88% of the particles that are nanometers in size. The mean of the 356 particles is 498.9 nm ± 597.4 nm. The size is bigger compared to a study done by Arzani et al. (2018) in which the mean size of the particle was 123 nm. The difference between the methods is the usage of chloroform compared to dichloromethane as the dissolution solvent of PLGA. Arzani et al. (2018) also stirred the solution of CUR-PLGA-PVA for a longer duration of 8–10 hours.

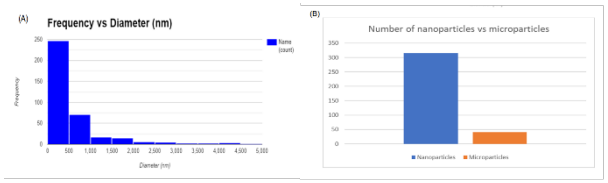

Fig. 2: Frequency of microparticles and nanoparticle molecules. (A) Frequency vs Diameter (in nm); (B) number of NPs vs microparticles.

Nanoparticle size and zeta potential measurement

The result of the size distribution by the intensity and zeta potential curcumin loaded PLGA are given in Figure 3 (a) and 3 (b) respectively. The Z-average of the curcumin-loaded PLGA is 444.7 nm with PDI 0.372. The zeta potential is -28.7 mV. ± 6.19 mV.

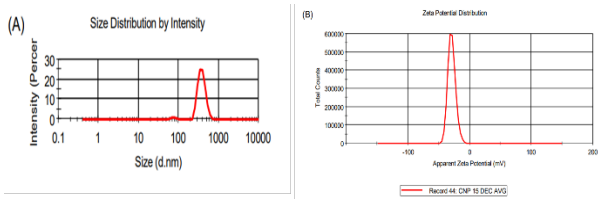

Fig. 3: Size distribution by intensity and zeta potential distribution for curcumin-loaded PLGA. (A) Size distribution by intensity; (B) Zeta potential distribution.

Arzani et al. (2018) obtained a polydispersity indice (PDI) of less than 0.27 and a zeta potential of about -40 mV due to the carboxyl group. Feczkó et al. (2011) found that the PDI from their distribution data ranged from 0.05 to 0.15 across different trials, typically centering around a PDI of 0.10. Mainardes and Evangelista (2005) achieved a PDI of 0.19 with a 5-minute sonication time. Tahara et al. (2017) obtained zeta potential of -40 mV and their particle size was 200 nm. The PDI was measured in this experiment as a parameter to show the homogeneity of nanoparticle droplet size and uniformity of particle size distribution. A PDI value of 0–1 indicates a uniform distribution; the closer to 0, the better (Arozal et al., 2020). The PDI value of the NPs in this experiment shows a result of 0.372. Meanwhile, the zeta potential of the NPs is -28.7 mV ± 6.19 mV. These readings are further from 0. One important metric that sheds light on the stability of colloidal dispersions, especially those containing NPs, is zeta potential. Because of the electrostatic

repulsion between the particles, NPs with larger absolute values of zeta potential—whether positive or negative—are generally more stable. It appears that the majority of the NPs' surface is negatively charged based on the negative zeta potential. An excellent degree of electrostatic repulsion between particles is indicated by a strong negative zeta potential, such as -28.7 mV ± 6.19 mV, which lowers the chance of aggregation.

Determination of Entrapment Efficiency and Drug Loading Capacity of Curcumin-loaded PLGA NPs.

The yield of the NPs is 99.01%. The initial drug used, which is curcumin, was 10 mg and after centrifugation, there were 5 mg of unentrapped drug.

$$\text{Entrapment Efficiency (\%)} = (10\text{mg}/5\text{mg}) \times 100\% = 50\%.$$

Meanwhile, drug loading capacity which is the percentage of the drug weight that is encapsulated about the total weight of the NPs is:

$$\text{Drug Loading Capacity (\%)} = (5\text{mg}/100\text{mg}) \times 100\% = 5\%$$

The entrapment efficiency of NPs shows a result of 50%. This indicates that only half of the curcumin was encapsulated in the PLGA particles while the remaining half might have been lost during loading or not incorporated properly. Drug-carrier interaction, solvent selection, preparation technique, and other formulation parameters could all have an impact on the entrapment efficiency. Meanwhile, the drug loading capacity of the NPs shows a result of 5%. This indicates that curcumin makes up 5% of the weight of all PLGA particles. A loading capacity that is deemed acceptable may vary depending on several factors, including the drug's solubility in the carrier and the formulation's stability.

Based on the study by Arzani et al. (2018), stated that increasing the amount of curcumin in the organic phase led to enhanced encapsulation efficiency (EE) and drug loading capacity (LC) of curcumin-loaded PLGA, with the increase being insignificant ($P > 0.05$). Specifically, raising the curcumin amount from 3 to 10 mg resulted in an increase in EE from 77.81% to 89.87% and LC from 7.86% to 10.53%. Utilizing low concentrations of curcumin is more suitable for achieving smaller particle sizes while maintaining an optimal level of drug entrapment (Arzani et al., 2018).

In vitro release of curcumin

To ascertain the release mechanism and quantity of curcumin released from the curcumin nanoparticle matrix system, the release of curcumin NPs was conducted using a dialysis membrane. The release test was conducted for 3 hours and repeated four times. From this, a graph was plotted (Figure 4a), and the mean of the reading has been made. From the absorbance data, the concentration of the released curcumin was determined which is shown in Figure 4b. From the figure it can be seen that the reading is not consistent, however it shows that the curcumin release had reached the plateau at 45 minutes and above.

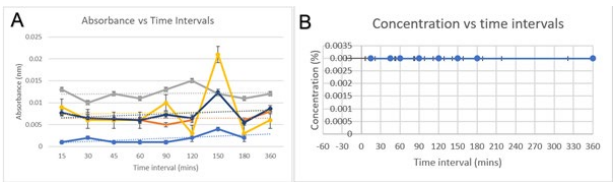

Fig. 4: In vitro release of curcumin NPs based on absorbance and concentration. (A) Absorbance vs time intervals; (B) Concentrations vs time intervals.

Based on the result of *in vitro* release using a dialysis membrane, it can be seen that only a small amount of curcumin was released from the dialysis membrane, which shows low reading. In this experiment, a dialysis tubing cellulose membrane (Sigma-Aldrich) with an average diameter of 16 mm and an average flat width of 25 mm has been used. The molecular weight cut-off of this dialysis membrane is 14000 Da which means the membrane will allow molecules smaller than 12,000–14,000 Da to pass through. The release of curcumin into the buffer may be affected by the size of curcumin, which could be too large for the pores of the dialysis membrane. One of the other reasons which affect the result is the low amount of curcumin being released into the buffer which makes the reading unreadable

by the UV-Vis spectrophotometry. To replicate the physiological circumstances of the small intestine, a pH of 6.8 has been selected. By selecting a buffer strength of 0.1 M, the ionic strength is guaranteed to be high enough to preserve the stability of the buffer solution. The interactions between molecules and the buffer's overall ability to maintain pH can both be impacted by the ionic strength. In an experiment done by (Halayqa & Domańska, 2014), they mention that larger NPs with higher encapsulation efficiency exhibit slower drug release for both drugs. The size of the NPs affects their dissolution rate, with smaller NPs showing faster dissolution due to increased surface area availability. The result of this experiment is not the same as Arzani et al. (2018) which managed to obtain the result of approximately 50% curcumin being released from the polymeric NPs in a rapid burst phase within the first 12 hours. Compared to Mogollon (2016), at room temperature (22°C), the release of curcumin occurs at a much slower rate, extending over approximately 96 hours. This slow release is primarily due to the reduced frequency of nanoparticle collisions at the organic-water interface. The percentage of accumulative release in the first 10 hours is around 25%.

Bioactivity of curcumin-loaded NPs.

The result from the effect of curcumin-loaded PLGA at different concentrations and the maximum cell viability of MCF-7 cells were studied at 48 hours. The relationship between the different concentrations of curcumin extract, curcumin NPs, negative control, and positive is given in Figure 5. There is a fluctuation that happened for every sample type except negative control. However, it can be seen that curcumin-loaded PLGA NPs showed a lower cell viability percentage compared to curcumin extract. There were no statistically significant differences between curcumin NPs and curcumin extract ($p > 0.05$). However, significant differences were observed between NPs and untreated cells ($p < 0.05$), as well as between NPs and cells treated with tamoxifen ($p < 0.05$). This aligns with findings from Arzani et al. (2018), indicating that curcumin-loaded PLGA is more effective than free curcumin. Similarly, a study by Tabatabaei Mirakabad et al. (2016) reported improved cell cytotoxicity with curcumin-loaded PLGA.

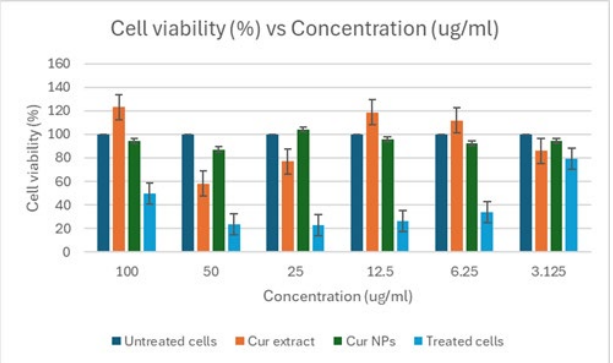

Fig. 5: Viability of cells vs series dilution against MCF-7

From the cytotoxicity study, it can be seen that the cell viability when treated with an extract of curcumin shows quite a variable reading compared to curcumin NPs. The cell viability of cytotoxic cells is lower compared to the viability of cells when treated with curcumin extract. This could be due to the hydrophobic characteristics of curcumin extract compared to curcumin-loaded PLGA NPs. Despite this cytotoxicity assay result, further testing needs to be done to show the cytotoxic effect of *C. xanthorrhiza*. There is currently little scientific information available regarding *C. xanthorrhiza's* mutagenic potential, genotoxicity, carcinogenicity, and even reproductive toxicity (Rahmat et al., 2021). Therefore, future research on the safety and effectiveness of *C. xanthorrhiza* extract and its active ingredients in specific therapeutic areas must be done.

Conclusion

The investigation aimed to formulate curcumin-loaded PLGA analyze the characteristics of curcumin-loaded PLGA and focus on their bioactivity. In this experiment, a single emulsion method was used to prepare curcumin-loaded PLGA. This process managed to produce NPs with an average size of 498 nm with a Z-average of 444.7 nm with PI 0.372 and a zeta potential of -28.7 mV ± 6.19 mV. However, the EE is quite low with 50% and DLC of 5%. The released study in vitro did not produce significant results as low concentrations of

release curcumin were detected. The bioactivity of curcumin-loaded PLGA shows lower viability of cells compared to curcumin extract which suggests that the PLGA formulation is more effective at promoting cell death. Further research needs to be done to further solidify the data on the release profile of curcumin from curcumin NPs and also an improved formulation needs to be done which increases the entrapment efficiency and drug loading capacity of curcumin inside the NPs.

Authors contributions

M.H.E: Investigation, Data Acquisition, Writing – Original Draft, Visualization. **M. T., D.S.:** Conceptualization, Methodology, Validation, Resources, Writing – Review & Editing, Supervision, Project administration, Funding acquisition. **S.**: Research materials, Resources. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

I have not used any AI tools or technologies to prepare this assessment.

References

Arozal, W., Louisa, M., Rahmat, D., Chendrana, P., & Sandhiutami, N. M. D. (2020). Development, Characterization and Pharmacokinetic Profile of Chitosan-Sodium Tripolyphosphate Nanoparticles Based Drug Delivery Systems for Curcumin. Advanced Pharmaceutical Bulletin, 11(1), 77–85. https://doi.org/10.34172/apb.2021.008

Dei Cas, M., & Ghidoni, R. (2019). Dietary Curcumin: Correlation between Bioavailability and Health Potential. Nutrients, 11(9), 2147. https://doi.org/10.3390/nu11092147

Feczkó, T., Tóth, J., Dósa, G., & Gyenis, J. (2011). Influence of process conditions on the mean size of PLGA nanoparticles. Chemical Engineering and Processing: Process Intensification, 50(8), 846–853.

Garms, B. C., Poli, H., Baggley, D., Han, F. Y., Whittaker, A. K., A, A., & Grøndahl, L. (2021). Evaluating the effect of synthesis, isolation, and characterisation variables on reported particle size and dispersity of drug loaded PLGA nanoparticles. Materials Advances, 2(17), 5657–5671. https://doi.org/10.1039/D1MA00410G

Guo, X., Zuo, X., Zhou, Z., Gu, Y., Zheng, H., Wang, X., Wang, G., Xu, C., & Wang, F. (2023). PLGA-Based Micro/Nanoparticles: An Overview of Their Applications in Respiratory Diseases. International Journal of Molecular Sciences, 24(5), 4333. https://doi.org/10.3390/ijms24054333

Gupta, S. C., Patchva, S., & Aggarwal, B. B. (2013). Therapeutic Roles of Curcumin: Lessons Learned from Clinical Trials. The AAPS Journal, 15(1), 195–218. https://doi.org/10.1208/s12248-012-9432-8

Halayqa, M., & Domańska, U. (2014). PLGA Biodegradable Nanoparticles Containing Perphenazine or Chlorpromazine Hydrochloride: Effect of Formulation and Release. International Journal of Molecular Sciences, 15(12), 23909–23923. https://doi.org/10.3390/ijms151223909

Hassanzadeh, K., Buccarello, L., Dragotto, J., Mohammadi, A., Corbo, M., & Feligioni, M. (2020). Obstacles against the Marketing of Curcumin as a Drug. International Journal of Molecular Sciences, 21(18), 6619.

https://doi.org/10.3390/ijms21186619

Hegde, M., Girisa, S., BharathwajChetty, B., Vishwa, R., & Kunnumakkara, A. B. (2023). Curcumin Formulations for Better Bioavailability: What We Learned from Clinical Trials Thus Far? ACS Omega, 8(12), 10713–10746. https://doi.org/10.1021/acsomega.2c07326

Karthikeyan, A., Senthil, N., & Min, T. (2020). Nanocurcumin: A Promising Candidate for Therapeutic Applications. Frontiers in Pharmacology, 11, 487. https://doi.org/10.3389/fphar.2020.00487

Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. Arabian Journal of Chemistry, 12(7), 908–931. https://doi.org/10.1016/j.arabjc.2017.05.011

Lanao, R. P. F., Jonker, A. M., Wolke, J. G. C., Jansen, J. A., Van Hest, J. C. M., & Leeuwenburgh, S. C. G. (2013). Physicochemical Properties and Applications of Poly(lactic-co-glycolic acid) for Use in Bone Regeneration. Tissue Engineering Part B: Reviews, 19(4), 380–390. https://doi.org/10.1089/ten.teb.2012.0443

Mainardes, R. M., & Evangelista, R. C. (2005). PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. International Journal of Pharmaceutics, 290(1-2), 137–144. doi:10.1016/j.ijpharm.2004.11.027

McCall, R. L., & Sirianni, R. W. (2013). PLGA Nanoparticles Formed by Single- or Double-emulsion with Vitamin E-TPGS. Journal of Visualized Experiments, 82, 51015. https://doi.org/10.3791/51015

Mogollon, C. (2016). In Vitro Release of Curcumin from Polymeric Nanoparticles Using Two-Phase System (Doctoral dissertation, University of Illinois at Chicago).

Operti, M. C., Bernhardt, A., Grimm, S., Engel, A., Figdor, C. G., & Tagit, O. (2021). PLGA-based nanomedicines manufacturing: Technologies overview and challenges in industrial scale-up. International Journal of Pharmaceutics, 605, 120807. https://doi.org/10.1016/j.ijpharm.2021.120807

Rahmat, E., Lee, J., & Kang, Y. (2021). Javanese Turmeric (Curcuma xanthorrhiza Roxb.): Ethnobotany, Phytochemistry, Biotechnology, and Pharmacological Activities. Evidence-Based Complementary and Alternative Medicine, 2021, 1–15. https://doi.org/10.1155/2021/9960813

Salleh, N. M., Ismail, S., & Ab Halim, M. (2016). Effects of Curcuma xanthorrhiza extracts and their constituents on phase ii drug-metabolizing enzymes activity. Pharmacognosy Research, 8(4), 309. https://doi.org/10.4103/0974-8490.188873

Tabatabaei Mirakabad, F.S., Akbaezadeh, A. Milani, M., Zarghami, N., Taheri-Anganeh, M., Zeighamian, V., … & Rahmati-Yamchi, M. (2016). A Comparison between the cytotoxic effects of pure curcumin and curcumin-loaded PLGA-PEG nanoparticles on the MCF-7 human breast cancer cell line. *Artificial cells, nanomedicine, and biotechnology*, *44*(1), 423-430.

Tahara, K., Karasawa, K., Onodera, R., & Takeuchi, H. (2017). Feasibility of drug delivery to the eye's posterior segment by topical instillation of PLGA nanoparticles. Asian Journal of Pharmaceutical Sciences, 12(4), 394–399. doi:10.1016/j.ajps.2017.03.002

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Infection Prevention and Control Knowledge among Health Sciences Students: A Cross-Sectional Study from Malaysia

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Abstract

Introduction: Infection prevention and control (IPC) is a crucial component of the healthcare system that demands adherence to standards to avoid and reduce the risk of infectious diseases spreading among patients, staff members, and visitors to healthcare institutions. The aim of this study was to assess IPC knowledge among undergraduate health sciences students and to identify the critical IPC components that need to be addressed. **Materials and method:** A cross-sectional online survey was conducted among 235 final-year health science students at International Islamic University Malaysia (IIUM), involving five faculties: the Faculty of Medicine, Dentistry, Pharmacy, Nursing, and Allied Health Science. A 45-item questionnaire was used to collect participants' sociodemographics (5 items) and explore their knowledge about IPC across six aspects (40 items). A score of > 24 (62%) indicates satisfactory knowledge. **Results:** The majority of the participants were female (74.9%), and 34% were from the Faculty of Pharmacy. Medicine students had the highest level of IPC knowledge with a mean score of 29.3 (n = 52), which was significantly different from Allied Health Science students (M = 25.6, n =55, $p < 0.001$). Other faculties students had similar IPC knowledge with no significant differences (Pharmacy: M = 27.5, n = 80; Nursing: M = 27.4, n = 29; Dentistry: M = 27.2, n = 19). IPC components that need to be improved are knowledge about sharp disposals and sharp injuries, as well as respiratory hygiene and cough etiquette. **Conclusion:** IPC knowledge was adequate among health science students at IIUM, although certain IPC components still require improvement. Additional IPC educational materials and workshops should be added to all faculties' syllabi to address this issue.

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Introduction

Infection prevention and control (IPC) measures have been implemented to prevent and control infectious diseases spread among patients, healthcare workers (HCWs), and visitors to healthcare institutions (Lowe et al., 2021). Hospital-acquired infections (HAIs) are among the major infectious causes of death and morbidity that pose a considerable risk to the health and safety of patients and HCWs, leading to rising healthcare costs (Li et al., 2022). The prevalence of nosocomial infections is estimated to be 7% and 10% in developed and developing countries, respectively. This is associated with prolonged hospital stay, reduced quality of life, and increased cost (Khan et al., 2017). The transmission of these illnesses occurs when HCWs handle and discard medical equipment, gather, process, and discard certain samples, and come into direct contact with patients (Alhassan et al., 2021). Therefore, infection prevention must be a top priority for healthcare facilities and organizations (WHO, 2016). Although hospitals have defined regulations and procedures, the IPC is inadequate. There is a global problem with a lack of standard precautions (SP) compliance, understanding, and attitudes among HCWs and health science students (Geberemariyam et al., 2018; Haile et al., 2017).

Education and training on IPC are becoming important, not only for healthcare professionals but also for students majoring in healthcare sciences. Undergraduate study is the ideal time to acquire the knowledge base and practical abilities necessary for future practice (Xiong et al., 2017; Ibrahim et al., 2016). Even so, it is unclear how effectively healthcare students understand IPC concepts. According to research conducted in 2012 in Saudi Arabia; IPC knowledge was limited and self-directed learning among medical students at King Faisal University. In addition, informal bedside practice served as the primary source of knowledge (Amin et al., 2013). However, a study at University in Albania found that students in four fields (medicine, physiotherapy, radiography, and nursing) had a moderate understanding of IPC and that classroom education was their primary source of information (Petrit et al., 2014). A recent study in Uganda revealed that health professional students at Makerere University possessed a high level of IPC knowledge; nevertheless, their IPC knowledge in various sections, such as hand hygiene, requires further improvement (Nalunkuma et al., 2021). Other study results showed that Saudi Arabian health science students had a satisfactory understanding of SPs and IPC, with no discernible gaps in knowledge between the sexes or between the Faculty of Medicine and other faculties (Khubrani et al., 2018).

The lack of knowledge and proper practice regarding IPC among HCWs may have an impact on healthcare students' behavioural practices when it comes to adopting IPC. Healthcare students should be equipped with IPC knowledge for a better practices during their clinical studies and future work. Therefore, in this study, we assessed the knowledge of healthcare students studying medicine, dentistry, pharmacy, nursing, and allied health science at International Islamic University of Malaysia (IIUM) about fundamental infection control measures, such as standard precautions, hand hygiene, respiratory hygiene, and the use of personal protective equipment. The assessment of IPC knowledge among healthcare students shall provide the basis for curriculum revision required to equip them with the necessary information and skills related to IPC, which in turn can improve their IPC practice during their future jobs as healthcare professionals.

Materials and methods

Study Design and Setting

This cross-sectional study was conducted at a Malaysian public university; i.e., IIUM – Kuantan Campus, from October 2022 to January 2023, using online approach.

Participants

The target sample was final year students in medicine, dentistry, pharmacy, nursing and allied health sciences undergraduate programs. Those who can read and understand English were included, and those who refused to participate were excluded from the study.

Study Questionnaire

The study self-administered questionnaire consisted of two parts. The first one included participants' sociodemographics (Table 1), while the second one was adopted from a recent study at Uganda's Makerere University's College of Health Science (Nalunkuma et al., 2021). The second part is divided into six sections (40 statements/questions) that measure participants' knowledge of several IPC aspects; i.e., general concept of IPC, hand hygiene, personal protective equipment (PPE), sharps disposal and sharp injuries, respiratory hygiene and cough etiquette, as well as care of healthcare providers (Table 2). A correct response to a statement or a question receives a score of one, whereas a false one receives a score of zero. A mean percentage score for each section was then obtained from the ratio between the number of correctly answered items and the total number of items. The questionnaire was piloted on 25 students to test its reliability among study population, which showed an acceptable reliability (Vaz et al., 2013), with a Cronbach's alpha values > 0.7 for all different IPC sections.

Study Size and Sampling

The minimum sample size determined for our study was 223, with a margin of error of 5%, and a level of confidence of 95%. The sample size was calculated using the Raosoft online sample size calculator (Raosoft Inc., Seattle, WA, USA). Convenience sampling was used to recruit participants.

Statistical Methods

Data collected were imported from Google Sheets to IBM SPSS software, version 21 for analysis. The descriptive statistics of means, standard deviations and percentages were used to summarize the responses of sociodemographics. Age, sex, study program, the sources of information on IPC and the number of these sources were all considered independent variables in the analysis. The percentage of all questions with the correct answers served as the dependent variable. Using multiple linear regression analysis, we investigated the relationship between sociodemographics and the mean percentage score of all questions. A $p < 0.05$ was set for the significance of the analysis.

Ethical Consideration

The study was approved by the IIUM Research Ethics Committee (IREC 2023-004). The questionnaire was prepared in Google Forms and a link was distributed to the students via text messages and class WhatsApp groups. The questionnaire started with an informational page that explained the study followed by consent section. Those who checked the "yes" box indicated their consent in participating in the study. There was no compensation for participation.

Results

Demographic Characteristics

A total of 235 students participated in the study, with a mean age of 22.98 ± 1.26. Most participants were female (176, 74.9%). Eighty out of 235 (34%) students were from the Faculty of Pharmacy. There were 199 out of 235 students (84.7%) reported that classroom instructions were their primary source of knowledge regarding IPC, while another 33.6% (n = 79) claimed they used three different sources (Table 1).

Knowledge of IPC Components

The statement "All body fluids except sweat should be viewed as sources of infection" had the lowest percentage of correct answers (67.2%; n = 158) in the section on the general concept of IPC. The least accurate response in the hand hygiene section was "Hand washing is indicated between tasks and procedures on the same patient," scoring only 45.5% (n = 107) of the total points. The least accurate response for the PPEs section was "PPEs are exclusively suitable to laboratory and cleaning staff for their protection," scoring 32.8% (n = 77). In the sharps disposal and sharp injuries section, the least correct answer was related to the statement "Soiled sharp objects should be shredded (cut into tiny pieces) before final disposal" with 10.2% (n = 24). For respiratory hygiene and cough etiquette, only half of the participants got all three correct answers (52.8%, n = 124). The least accurate response for the section of care for healthcare providers was on "The risk for a health provider to acquire HIV infection following a needle-stick injury is less than 0.5%," coming in at 16.6% (n = 39) correct response (Table 2).

Table 1: Demographic characteristics of the participants (N = 235)

	Frequency (n)	Percentage (%)
Sex		
Male	59	25.1
Female	176	74.9
Study Program		
Faculty of Medicine	52	22.1
Faculty of Dentistry	19	8.1
Faculty of Pharmacy	80	34
Faculty of Allied Health Science	55	23.4
Faculty of Nursing	29	12.3
Sources of Information on IPC		
Self-learning	148	63
Informal practical learning	142	60.4
Formal curricular teaching	199	84.7
Infection control courses	105	44.7
Media and/or internet	75	31.9
Number of sources of information		
1	24	10.2
2	71	30.2
3	79	33.6
4	39	16.6
5	22	9.4

IPC: Infection prevention and control

Table 2: Proportions of correct responses in the knowledge domain of various IPC aspects (N = 235)

	Proportion of Correct Response (n)
Section A: General Concept of Infection IPC	
The main goal of infection control is: (1 option)	94.9 (223)
Definition of standard precautions: (1 option)	97.4 (229)
All patients are sources of infections regardless of their diagnoses. (true)	75.3 (177)
All body fluids except sweat should be viewed as sources of infection. (true)	67.2 (158)
Section B: Hand Hygiene	
Hand washing minimizes microorganisms acquired on the hands if hands are soiled (true)	95.3 (224)
Handwashing reduces the incidence of healthcare-related infections (true)	97.4 (229)
In standard handwashing: the minimum duration should be. . . (1 option)	74.9 (176)

Hand decontamination: includes washing the hands with antiseptic soap for 30 seconds (1 option)	74.9 (176)
Alcohol hand rub substitutes hand washing even if the hands are soiled (false)	55.3 (130)
Hand washing is indicated between tasks and procedures on the same patient (true)	45.5 (107)
The use of gloves replaces the need for handwashing (false)	77 (181)
Hand washing is indicated after removal of gloves (true)	93.2 (219)
Hand washing is needed with patients with respiratory infections including COVID-19 (true)	98.7 (232)
Section C: Personal Protective Equipment (PPE)	
PPEs such as masks and head caps provide protective barriers against infection (true)	97.4 (229)
Use of PPEs eliminates the risk of acquiring occupational infections (true)	86 (202)
PPEs are exclusively suitable to laboratory and cleaning staff for their protection (false)	32.8 (77)
PPEs should be used only whenever there is contact with blood (false)	91.1 (214)
Gloves and masks can be re-used after proper cleaning (false)	96.6 (227)
Used PPEs are to be discarded through regular dust bins (false)	90.6 (213)
Gloves should be changed between different procedures on the same patient (true)	41.7 (98)
Masks made of cotton or gauze are most protective (false)	44.7 (105)
Masks and gloves can be re-used if dealing with same patient (false)	53.6 (126)
Section D: Sharps disposal and sharp injuries	
Used needles should be recapped after use to prevent injuries (false)	38.7 (91)
Used needles should be bent after use to prevent injuries (false)	77.9 (183)
Sharps container is labelled with. . .(1 option)	61.7 (145)
Soiled sharps objects should be shredded (cut into tiny pieces) before final disposal (true)	10.2 (24)
Sharps injuries should be managed with no need of reporting (false)	88.5 (208)
Needle-stick injuries are the least commonly encountered in general practice (false)	72.8 (171)
Post-exposure prophylaxis is used for managing needle stick injuries from an HIV-infected patient (true)	60.9 (143)
Immediate management of sharps injuries includes. . . (1 option)	51.5 (121)
Section E: Respiratory hygiene and cough etiquette	
Cough/sneeze on a disposable napkin and wash your hands (true)	1 correct: 12.3 (29)
Cough/sneeze over the shoulder if a napkin is not available (true)	2 corrects: 34.9 (82)

Keep a distance of 3 feet from others when coughing (true)	3 corrects: 52.8 (124)
Wipe your hands on the inside of your white coat after you cough or sneeze (false)	
Section F: Care of healthcare providers	
Immunization history of health care providers should be obtained before recruitment (true)	96.6 (227)
The risk for a health provider to acquire HIV infection after a needle-stick injury is. . . (option)	16.6 (39)
Post-exposure immunization prevents the risk of hepatitis B infection following exposure (true)	52.3 (123)
For the prevention of hepatitis B, immunizations are recommended for all healthcare workers (true)	91.1 (214)
Following exposure to a patient with flu, antibiotics are required for the prevention of infection (false)	57.9 (136)
Health providers with the highest risk of exposure to tuberculosis include radiologists (true)	45.5 (107)

IPC: Infection prevention and control; *PPE:* Personal protective equipment

Table 3: Mean percentage score and standard deviation of total scores of each IPC section (N = 235)

Total score of:	**Mean percentage score (SD)**
General Concept of IPC	83.7 % ± 73.3%
Hand Hygiene	79.2% ± 19.1%
PPE	70.5% ± 26.6%
Sharps disposal and Sharp Injuries	57.8% ± 24.7%
Respiratory hygiene and cough etiquette	57.8% ± 20.3%
Care of healthcare providers	60.0% ± 29.9%
The average for all total scores for each parameter	68.2% ± 5.52%

SD: Standard deviation; *IPC:* Infection prevention and control; *PPE:* Personal protective equipment

Table 3 shows that mean percentage score of sharp disposals and sharp injuries section (57.8% ± 24.7%), as well as respiratory hygiene and cough etiquette section (57.8% ± 20.3%), were the lowest of all IPC sections.

Factors Affecting Health Science Students' Score of Correct Answers

Multiple regression analysis was run to assess the relationship between students' socio-demographics and the total score of correct answers for all questions. There was a significant relationship between the number of information sources and the total score of IPC knowledge (p = 0.03). There was no significant relationship between other sociodemographics and the score of IPC knowledge.

We also run one-way ANOVA analysis to gauge differences in the mean total score among different study programs. Faculty of Medicine had the highest mean total score (29.3 ± 3.48), while faculty of Allied Health Science had the lowest (25.6 ± 4.24). Other faculties had similar mean total scores; i.e., Pharmacy: M = 27.5 ± 3.56; Nursing: M = 27.4 ± 5.51; Dentistry: M = 27.2 ± 2.92. Post Hoc test revealed a statistically significant difference only between faculties of Medicine and Allied Health Science (p < 0.001).

Discussion

In this study, we assessed knowledge of IPC among final year health science students from Faculties of Medicine, Dentistry, Pharmacy, Allied Health Science and Nursing at a Malaysian public university, and found that students from Faculty of Medicine had the highest level of knowledge of IPC, and that among all faculties, students IPC knowledge needs to be improved.

We discovered that students at the Faculty of Medicine had better knowledge of the various components of IPC than students at other faculties, which was significantly better than those from Faculty of Allied Health Sciences. In comparison, a study conducted at King Saud bin Abdulaziz University for Health Sciences found that nursing students had the highest percentage of people displaying appropriate knowledge (Geberemariyam et al., 2018). Our findings showed that although the health science students' IPC knowledge was deemed good, there were knowledge gaps in sharps disposal and sharps injuries, respiratory hygiene, and cough etiquette. Nevertheless, Khubrani and colleagues reported that respiratory hygiene, cough etiquette, and care of healthcare providers were the least well-known sections among their participants (Khubrani et al., 2018). Although the lack of understanding of the indication of handwashing between task and procedure on the same patient was evident, all 235 students in the present study demonstrated a fair understanding of hand hygiene. In contrast, a study by Makerere University Faculty of Health Science revealed that only 60.4% of participants knew that hand washing is necessary between tasks, even when attending to the same patient (Haile et al., 2017). This suggests that a lack of understanding about the importance of handwashing increases the risk of infection and illness transmission by healthcare professionals among patients.

Most of our participants (67.2%, n = 158) correctly identified all body fluids as sources of infection except for sweat. However, this question received the lowest correct responses compared to the other questions in Section A, which was in line with a study conducted in Uganda (Haile et al., 2017). This demonstrates that students from different countries lack appropriate awareness of body fluids as a medium of infection transmission between patients and healthcare professionals. Of the 235 participants, 77 (32.8%) believed that PPEs were only appropriate for laboratory and cleaning staff which is untrue. In contrast, Nalunkuma and colleagues found that only 46.53% of the participants were aware that PPEs might also be used to lessen exposure to dangers that could result in significant illnesses and injuries in addition to being utilized in laboratories and for cleaning (Nalunkuma et al., 2021). The chance for a healthcare professional to contract human deficiency virus (HIV) following a needle-stick

injury question had the fewest accurate answers in the section on the care of healthcare providers, with just 39 students (16.6%) answering it correctly.

The health science students involved in this study had the lowest knowledge regarding sharps disposal, sharp injuries, respiratory hygiene, and cough etiquette, scoring only 57.8% for both sections. This partially contradicts the findings from a related study conducted among health science students in Uganda (Haile et al., 2017). where respiratory hygiene and cough etiquette received a higher score (80.2%), while disposal of sharps had the lowest score (61.55%), which is consistent with our study. In contrast, our study participants scored higher in the care of healthcare providers section compared to Uganda study participants, which could be a result of focusing on healthcare provider care in the curricula of our participants' study programs. The results of our study also showed that among all study programs, Faculty of Medicine has the highest level of IPC knowledge, followed by Faculty of Nursing, Faculty of Pharmacy, Faculty of Dentistry, and Faculty of Allied Health Science, which has the lowest score. These results align with research among Saudi Arabian students (Khubrani et al., 2018), where it was discovered that students who spend more time in clinical and medical environments are likely to have higher knowledge about IPC.

The inferior quality of healthcare delivery results has been attributed to healthcare professionals' ignorance of IPC (Khubrani et al., 2018). Numerous studies that demonstrate the need for more education and training support the fact that the majority of healthcare professionals admitted that they did not receive any training or orientation on IPC in their undergrad studies. They were also unsure if they had received insufficient knowledge and lower academic education and training on IPC (Alhassan et al., 2021). To ensure better performance in healthcare delivery as future healthcare professionals, health science students must be equipped with sufficient IPC knowledge (Ibrahim et al., 2016).

There were a few limitations to this study. The questionnaire used was not evenly distributed to all study programs, and the data collection instrument restricts the observation of behavior, skills, and student compliance during the survey. Future studies could be conducted with an equally recruited participants among different study programs for a more accurate representation of the population. Data acquisition from other universities could also be done for better analysis, but it requires more time and funding. Different syllabi and exposure to IPC knowledge across universities also restrict the generalization of results.

Conclusion

IPC demands adherence to standards to avoid and reduce the risk of infectious diseases spreading among health practitioners, residents and visitors. This makes IPC a vital component of healthcare system. Being future healthcare practitioners, health sciences students should be equipped by proper IPC knowledge. Our findings showed that although health sciences students have a good IPC knowledge, a few components of IPC need to be addressed; i.e., sharps disposal and sharps injuries, as well as respiratory hygiene and cough etiquette.

To improve IPC knowledge among health sciences students, educators should be aware about the importance of incorporating the IPC-related materials in the syllabi of different health sciences programs, with the availability of equipments needed for IPC and SPs lessons or training. Health educators should also provide formal clinical skills training, which is crucial; else, safety may rely on accidental learning from other healthcare professional (Grundgeiger et al., 2023). Health educator could also apply different approaches to facilitate their students' learning such as seminars and interactive workshops, which proofed to improve students knowledge (Mukurunge et al., 2021). Hands-on blended with e-learning might also be considered in IPC knowledge obtainment and had been resulted in improved knowledge while addressing the

problem of different learning paces (Grundgeiger et al., 2023).

Authors contributions

Conceptualization: A.R.F.N.; Data curation: H.Z.S.; Methodology/formal analysis/validation: A.F.N., H.Z.S. Project administration: A.R.F.N.; Writing–original draft: H.Z.S.; Writing–review & editing: A.R.F.N., M.E.A., T.E.S., M.H.E., N.H.M.T., A.M.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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References

Alhassan, A. R., Kuugbee, E. D., & Der, E. M. (2021). Surgical healthcare workers knowledge and attitude on infection prevention and control: A case of tamale teaching hospital, Ghana. *Canadian Journal of Infectious Diseases and Medical Microbiology*, *2021*. https://doi.org/10.1155/2021/6619768

Amin, T. T., Noaim, K. I. A., Saad, M. a. B., Malhm, T. a. A., Mulhim, A. a. A., & Awas, M. a. A. (2013). Standard precautions and infection control, Medical Students' knowledge and behavior at a Saudi University: the need for change. *Global Journal of Health Science*, *5*(4). https://doi.org/10.5539/gjhs.v5n4p114

Biberaj, P., Gega, M., & Bimi, I. (2014). Knowledge and source of information among health care students on nosocomial infections. IJHSSE, 1(7), 46-51. https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=406f4cae42118050a0ba8a72b2b14d7575f5c85f

Geberemariyam, B. S., Donka, G. M., & Wordofa, B. (2018). Assessment of knowledge and practices of healthcare workers towards infection prevention and associated factors in healthcare facilities of West Arsi District, Southeast Ethiopia: a facility-based cross-sectional study. *Archives of Public Health*, *76*(1). https://doi.org/10.1186/s13690-018-0314-0

Grundgeiger, T., Ertle, F., Diethei, D., Mengelkamp, C., & Held, V. (2022). Improving procedural skills acquisition of students during medical device training: experiments on e-Learning vs. e-Learning with hands-on. Advances in Health Sciences Education, 28(1), 127–146. https://doi.org/10.1007/s10459-022-10148-0

Haile, T. G., Engeda, E. H., & Abdo, A. A. (2017). Compliance with Standard Precautions and Associated Factors among Healthcare Workers in Gondar University Comprehensive Specialized Hospital, Northwest Ethiopia. *Journal of Environmental and Public Health*, *2017*, 1–8. https://doi.org/10.1155/2017/2050635

Ibrahim, A. A., & Elshafie, S. S. (2016). Knowledge, awareness, and attitude regarding infection prevention and control among medical students: a call for educational intervention. *Advances in Medical Education and Practice*, *Volume 7*, 505–510. https://doi.org/10.2147/amep.s109830

Khan, H. A., Baig, F. K., & Mehboob, R. (2017). Nosocomial infections: Epidemiology,

prevention, control and surveillance. *Asian Pacific Journal of Tropical Biomedicine*, *7*(5), 478-482. https://doi.org/10.1016/j.apjtb.2017.01.019

Khubrani, A. M., Albesher, M., Alkahtani, A., Alamri, F., Alshamrani, M. M., & Masuadi, E. (2018). Knowledge and information sources on standard precautions and infection control of health sciences students at King Saud bin Abdulaziz University for Health Sciences, Saudi Arabia, Riyadh. *Journal of Infection and Public Health*, *11*(4), 546–549. https://doi.org/10.1016/j.jiph.2017.10.013

Li, P., Li, Y., Zhang, Y., Bao, J., Yuan, R., Lan, H., & Sun, M. (2022). Economic burden attributable to healthcare-associated infections in tertiary public hospitals of Central China: a multi-centre case-control study. *Epidemiology & Infection*, *150*, e155. https://doi.org/10.1017/S0950268822001340

Lowe, H., Woodd, S., Lange, I.L., Janjanin, S., Barnet, J., & Graham, W. (2021). Challenges and opportunities for infection prevention and control in hospitals in conflict-affected settings: a qualitative study. *Conflict and health*, 15(94) 1-10. https://doi.org/10.1186/s13031-021-00428-8

Mukurunge, E., Reid, M., Fichardt, A., & Nel, M. (2021). Interactive workshops as a learning and teaching method for primary healthcare nurses. *Health SA Gesondheid*, *26*. https://doi.org/10.4102/hsag.v26i0.1643

Nalunkuma, R., Nkalubo, J., & Abila, D. B. (2021). Knowledge on Infection Prevention and Control and associated factors among undergraduate health professional students at Makerere University College of Health Sciences, Uganda. *PloS One*, *16*(8), e0255984. https://doi.org/10.1371/journal.pone.0255984

Vaz, S., Parsons, R., Passmore, A., Andreou, P., & Falkmer, T. (2013). Internal consistency, Test–Retest reliability and measurement error of the Self-Report version of the social Skills rating System in a sample of Australian adolescents. *PloS One*, *8*(9), e73924. https://doi.org/10.1371/journal.pone.0073924

World Health Organization. (2016). *Core components: Guideline recommendation*. Guidelines on Core Components of Infection Prevention and Control Programmes at the National and Acute Health Care Facility Level - NCBI Bookshelf. https://www.ncbi.nlm.nih.gov/books/NBK401782/

Xiong, P., Zhang, J., Wang, X., Wu, T. L., & Hall, B. J. (2017). Effects of a mixed media education intervention program on increasing knowledge, attitude, and compliance with standard precautions among nursing students: A randomized controlled trial. *American Journal of Infection Control*, *45*(4), 389–395. https://doi.org/10.1016/j.ajic.2016.11.006

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Avocado (*Persea americana* Mill.) bioactive compounds - extraction method, chemical compositions and cosmeceutical applications: A scoping review)

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Abstract

The cosmeceutical industry has been blooming over the years, necessitating a demand for safe and effective options. Fruit bioactive compounds are reported as safe for human health and broadly effective alternatives with less adverse effects. Avocado (*Persea americana* Mill.) is a tropical fruit rich in phytonutrients and lipid-soluble bioactive compounds. These compounds have been reported to have various potential health benefits, including improving skin health. This scoping review investigated the bioactive compounds of avocados that were reported to confer beneficial activities on the skin. Published data between August 1982 till February 2022 were extracted from Ovid Medline, Scopus, Pubmed, SciFinder and Web of Science. A total of 307 published articles were identified using the search terms, of which 31 full articles were reviewed and appraised in this synthesis. Results: This comprehensive scoping review examined the cosmeceutical activities of bioactive phytochemicals found in avocado (*Persea americana* Mill.) outlining their mechanisms of action. The review highlighted the antioxidant, antimicrobial, anti-inflammatory, wound healing, anti-tyrosinase, and anti-aging properties of avocado extracts. Acetone extracts, especially from seeds, showed the highest antioxidant capacity and were also effective in antimicrobial activities. Methanol extracts demonstrated significant anti-inflammatory effects. Furthermore, bioactive compounds from avocados were found to enhance wound healing and anti-aging effects, such as increasing collagen production and improving skin hydration and elasticity. This scoping review provides a comprehensive collection of evidence and critically appraises recent literature on bioactive compounds of Avocado and extraction solvents and potential cosmeceutical applications.

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Introduction

Cosmeceuticals are defined as cosmetics with active compounds that has positive therapeutic qualities on the skin (Husein El Hadmed & Castillo, 2016). These products aim to enhance skin appearance and health while protecting against environmental damage from UV radiation, pollution, and oxidative stress (Husein El Hadmed & Castillo, 2016). In recent years, the cosmeceutical sector has been experiencing significant growth, with an annual increase in consumer spending on cosmetics aimed at delaying or reversing skin aging. Research suggests that there has been a 33% rise in expenditure within the anti-aging industry every year. Notably, there has been a shift towards natural bioactive ingredients in the cosmeceutical business, largely due to concerns about the ineffectiveness of synthetic cosmetics. This trend reflects a growing interest in products that are perceived as more beneficial for skin health (Smit et al., 2009; Yon et al., 2023).

Plant-derived bioactive compounds, such as polyphenols, phytosterols, biogenic amines, and carotenoids (Samtiya et al., 2021), have been found to have cosmeceutical benefits including moisturizing, revitalizing, anti-aging, UV protection, and preventing skin-related disorders. These compounds are highly sought-after as active ingredients in the cosmeceuticals industry (Romes et al., 2021). The demand for plant-derived bioactive components is driven by their non-artificial synthetic chemical properties, which are known to be less irritating to the skin compared to synthetic chemicals (Lagoa et al., 2020; Puglia & Santonocito, 2019). Other examples such as butylated hydroxyanisole (BHT) and butylated hydroxytoluene (BHA) have been restricted due to potential carcinogenicity (Pandey & Kumar, 2021).

Research has indicated that consuming bioactive antioxidants through food can offer cosmeceutical advantages. These include safeguarding skin cells, defending against dryness and environmental harm, and maintaining adequate moisture levels for optimal function (Nilforoushzadeh et al., 2018). Studies have also emphasized the benefits of antioxidants in providing nutrients for healthy skin, reducing wrinkles, and enhancing skin brightness, texture, and tone (Thiyagarasaiyar et al., 2020).

Avocado, also known as *Persea americana* Mill., is a plant from the Lauraceae family (Lister et al., 2021). It is grown worldwide, primarily in tropical and subtropical regions with warm and moderate conditions (García-Villegas et al., 2022). There is a diverse variety of avocado species, which includes Hass, Fuerte, Gwen, Bacon, and Reed, among many others, which can be categorised into main groups of: West Indian, West Indian-Guatemalan hybrid, Guatemalan and Mexican (Melgar et al., 2018; Wang et al., 2010) .

Avocado has gained an increased worldwide interest in recent years due to its high nutritional value (Moldovan et al., 2021). It is a popular fruit known for its unique taste, year-round availability, and excellent source of nutrients such as saponins, alkaloids, polyphenols, tannins, and flavonoids found in the fruit, seeds, and leaves. These phytonutrients contribute to antioxidant, antimicrobial, anti-inflammatory, wound-healing, and anti-aging properties (Lister et al., 2021). Consumption of avocado has also been linked to improved skin health due to its high bioavailability of carotenoids, lutein, and zeaxanthin, which can help prevent damage to the skin from ultraviolet (UV) and visible radiation (Dreher & Davenport, 2013)

Different parts of the plant are reported to confer specific benefits. Avocado oil is known to be rich in unsaturated fatty acids, lecithin, minerals, β-sitosterol, β-carotene, and vitamins A, C, D, and E. This makes it an excellent choice for moisturizing the skin when used topically (Lin et al., 2017). Studies have shown that avocado oil may help in reducing wrinkles, stretch marks, promoting wound healing, and treating psoriasis by aiding in the regeneration of the epidermis (Ana Paula de Oliveira et al., 2013; Poljšak et al., 2020). Consequently, multiple studies have been conducted on avocados to support their use in cosmeceuticals (Lister et al., 2021).

This scoping review critically appraises and summarises the published data on avocado

bioactive compounds and the solvents used for their extraction, exploring their potential applications in the cosmetics industry. Additionally, the review provided a critical assessment of various extraction solvents, including methanol, ethanol, hexane, chloroform, and others, utilized for isolating bioactive compounds from avocados.

Materials and methods

This scoping review aimed to evaluate the most recent and representative information on the bioactive phytochemicals of functional extracts from *Persea americana* Mill. This scoping review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (Tricco et al., 2018) .

Search Strategy

The present scoping review was carried out using the electronic databases: Ovid MEDLINE, Scopus, Pubmed, SciFinder and Web of Science. We retrieved published data from August 1982 to January 2022. Specific keywords "avocado" OR "*Persea americana* Mill." AND "skin" AND "cosmetics" were used. The same strategy was used for all the databases with adaptations, as appropriate. Next, the list of studies was completed by searching the bibliographies of the selected publications and implementing the inclusion and exclusion criteria in each case. Results were limited to the English language. All the research articles had been published in peer-reviewed journals, and those with fully accessible texts were selected.

Inclusion Criteria

Studies reporting on 1) *Persea americana* Mill. parts, including leaves, pulp, seed, and peel, and 2) its nutrients or bio compounds were included.

Exclusion Criteria

The exclusion criteria were as follows: a) studies published in languages other than English were excluded, b) Patents, literature reviews, and systematic reviews, c) Data from non-open access journal articles or partially accessed (abstract only) articles.

Data Extraction

The two-steps selection was carried out: 1) non-eligible article were removed by screening the titles and abstract, and 2) three authors, XNL., LLY., and WYL., independently screened titles, abstracts, and full-text articles according to the stated inclusion criteria and data availability. The data were extracted from main articles and their supplementary materials into an excel spreadsheet. Two other authors (SM., BHG.) then review the selected data. Disagreements were resolved via discussion and consultation, which were then amended. The following data were extracted from each study: 1) Study characteristics: author name, year of publication, country of study, total phenolic content of extracts, total antioxidant capacity, wound healing properties, antibacterial activity, anti-tyrosinase activity and anti-inflammatory activities. A reference manager (EndNote X9, Thompson Reuters, Philadelphia, PA, USA) was used to import the list of references from all databases, where duplicates were then removed.

Study selection process

This review has extracted and summarised findings from published data on the cosmeceutical application of avocado and its bioactive compounds. A total of 307 published articles were identified through literature screening. The duplicates (n = 94) and articles that were not related to the subject of this review (n = 182) were removed, resulting in 31 articles remaining. Figure 1 is the illustrative flow chart showing article extraction and filtering.

Of the 31 studies included in this systematic review, 11 studies evaluated antioxidant capacity of avocado (Ekom & Kuete, 2022; Ferreira da Vinha et al., 2013; Forero-Doria et al., 2017; Hürkul et al., 2021; Kosińska et al., 2012; Melgar et al., 2018; Nguyen et al., 2021; Paoletti et al., 2010; Rodríguez-Carpena et al., 2011), five studies on antimicrobial activity (Donnarumma et al., 2007; Ekom & Kuete, 2022; Melgar et al., 2018; Nguyen et al., 2021; Paoletti et al., 2010; Rodríguez-Carpena et al., 2011), six on anti-inflammatory properties (Borghi et al., 2015; Hürkul et al., 2021; Rosenblat et al., 2011; Sharquie

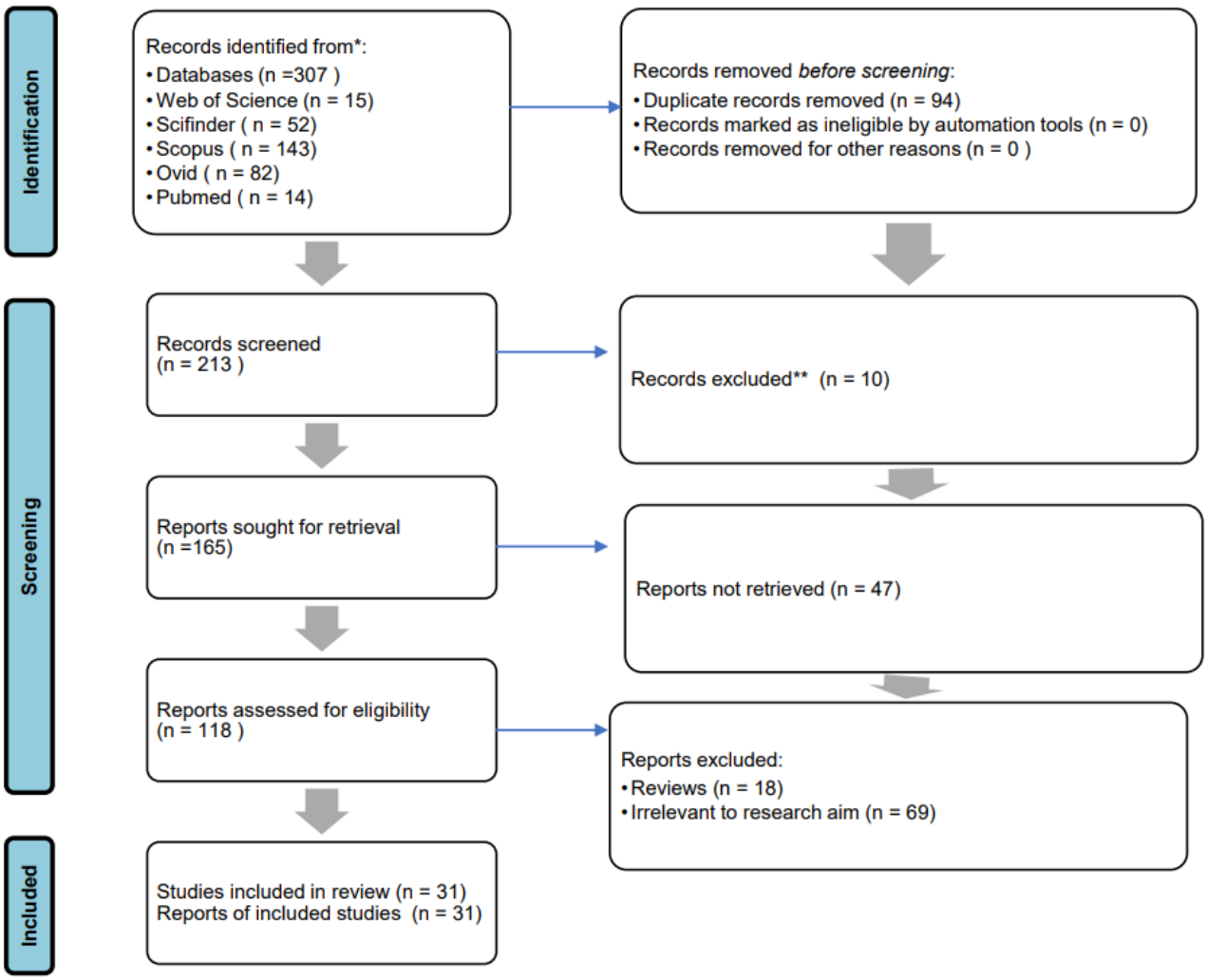

Fig. 1: Schematic illustration of article screening and filtering.

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et al., 2012) , six on wound healing properties (Alves et al., 2019; Ana Paula de Oliveira et al., 2013; Ekom & Kuete, 2022; Oryan et al., 2015; Sichani et al., 2021), two on anti-tyrosinase properties (Hürkul et al., 2021; Laksmiani et al., 2020) and four studies discussed its anti-ageing properties (Susanne M. Henning et al., 2022; Lister et al., 2021; Moldovan et al., 2021; Naeimifar et al., 2020). Figure 2 is an avocado fruit structure and its cosmeceutical benefits appraised in this review.

Results and discussion

This scoping review aimed to provide a complete description of the cosmeceutical's activities of bioactive phytochemicals of *Persea americana* Mill. The mechanisms of action through which these bioactive compounds or functional extracts exhibit cosmeceutical activities were defined.

After conducting an exhaustive screening, 31 selected articles were extracted and appraised. The findings were systematically categorized into three main areas: bioactive phytochemicals and their cosmeceutical benefits, extraction methods of bioactive phytochemicals, and in vitro and in vivo studies on these compounds.

The review highlights the cosmeceutical benefits of bioactive phytochemicals, including their

antioxidant capacity, anti-inflammatory properties, wound-healing potential, anti-tyrosinase activity, and anti-ageing effects (Table 1).

Antioxidant capacity

The avocado's antioxidant capacity correlates with total phenolic content and procyanidins. Studies included in this synthesis showed that different extracts were used to obtain active phytochemicals of avocados (Table 1). Acetone extracts exhibited the most significant antioxidant capacity, followed by Methanol and ethyl acetate (Rodríguez-Carpena et al., 2011). Various assays were also used to obtain values to compare the antioxidant capacity among parts of the avocado or types of cultivars, mainly the DPPH, ORAC, ABTS and TBARS assays. Comparing IC50 and EC50 values from these assays showed that the Tonnage cultivar possesses the highest antioxidant activity, followed by Simmonds, Hass, Booth 8, Choquette, Booth 7, Loretta and Slimcado. In terms of avocado parts, seeds generally contribute the most to the antioxidant capacity compared to the peel and pulp.

According to a study by Melgar and colleagues (2018), the methanol extract of Hass avocado seeds demonstrated more significant antimicrobial activity compared to the acetone and diethyl ether extracts. The study found that avocado seeds exhibited higher antimicrobial activity than peels, as indicated by greater MIC values and lower MBC values in antibacterial and antifungal assays.

Additionally, Hurkul and colleagues (2021) reported that the HRBC membrane stabilization assay was the primary method used to evaluate the anti-inflammatory properties of avocado. The results indicated that methanol extract produced lower IC50 values than the N-hexane extract, suggesting greater anti-inflammatory activity. However, the study found contradictory results when comparing the anti-inflammatory activity of different parts of the avocado, such as peels, seeds, and pulp. The study also discussed the anti-tyrosinase capacity of avocado, noting that the highest anti-tyrosinase activity was associated with avocado peel extracted by N-hexane.

Different extraction solvents at various concentrations result in different extraction efficiencies where the composition of phenolic compounds responsible for antioxidant properties is affected (Table 1). Acetone extracts exhibited the most significant antioxidant capacity, followed by methanol and ethyl acetate (Rafique & Akhtar, 2018; Rodríguez-Carpena et al., 2011). The DPPH assay in Rodriguez-Carpena and colleagues (2011) showed that Hass avocado seeds extracted with acetone produced an In Vitro antioxidant activity of 130.26±36.80 mmol Trolox/g fresh matter. In contrast, methanol extract exhibited 66.24±24.84 mmol Trolox/g fresh matter, and ethyl acetate exhibited 17.78±4.34 mmol Trolox/g fresh matter.

Rafique and Akhtar (2018) state that acetone extracts are better antioxidants, with 80% activity in 60% acetone and 10% methanol compared to 70% methanol. However, the ratio of acetone has been shown to affect the antioxidant capacity of avocado extracts. 50% acetone extract will achieve a greater antioxidant capacity compared to 70% acetone due to the higher phenolic content achieved. DPPH and ABTS assays can be used to obtain IC50 values, where the lower the values, the greater the antioxidant capacity. Whereas another study comparing ultrasound-assisted batch extraction (UABE) and ultrasound-assisted continuous extraction (UACE) showed no significant differences in antioxidant capacity. Differences were only noticed in the extraction times where UACE reaches equilibrium up to 53% faster than UABE (Oryan et al., 2015)

The results obtained by Vinha and colleagues (2013) were in agreement with other studies, which stated that Hass avocado seeds exhibited the highest antioxidant capacity of 43%, followed by peels (35%) and pulp (23%). The study by Melger and colleagues (2018) reported that Bacon avocados' seeds, peels and pulps extracted with methanol solution and tested via DPPH and ABTS assay, showed IC50 values of seeds to be the lowest (4.17±0.04 mg/mL and 0.03±0.01 mg/mL) followed by peels (5.25±0.05 mg/mL and 0.06±0.02 mg/mL).

Pulps showed the highest values of 11.34±0.11 mg/mL and 0.65±0.08 mg/mL. Interestingly, the

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Antioxidant	Ethyl acetate	Hass	Seed	- TPC - Flavonols - Procyanidins - Catechins - OH-B - OH-C	N/A	CUPRAC	58.00 ±15.55	(Rodríguez-Carpena et al., 2011)
						DPPH	17.78 ± 4.34	
						ABTS	21.57 ± 7.51	
			Peel			CUPRAC	56.40 ± 21.19	
						DPPH	17.85 ± 7.07	
						ABTS	16.12 ± 6.98	
			Pulp			CUPRAC	2.48 ± 0.33	
						DPPH	0.37 ± 0.07	
						ABTS	0.64 ± 0.10	
		Fuerte	Seed			CUPRAC	96.09 ± 27.76	
						DPPH	27.80 ±10.16	
						ABTS	38.15 ±12.78	
			Peel			CUPRAC	103.68±26.69	
						DPPH	35.18 ± 12.56	
						ABTS	34.82 ±12.61	
			Pulp			CUPRAC	2.44 ± 0.65	
						DPPH	0.23 ± 0.07	
						ABTS	0.56 ± 0.11	

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review (cont.)

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Antioxidant	Acetone	Hass	Seed	- TPC - Flavonols - Procyanidins - Catechins - OH-B - OH-C	N/A	CUPRAC	275.36±59.09	(Rodríguez-Carpena et al., 2011)
						DPPH	130.26±36.80	
						ABTS	158.29±26.27	
			Peel			CUPRAC	218.04±42.42	
						DPPH	88.94±48.22	
						ABTS	103.75±44.49	
			Pulp			CUPRAC	1.63±0.39	
						DPPH	0.33±0.07	
						ABTS	0.84±0.24	
		Fuerte	Seed			CUPRAC	353.43±75.83	
						DPPH	167.50±42.08	
						ABTS	194.80±44.69	
			Peel			CUPRAC	456.24±77.07	
						DPPH	199.61±33.15	
						ABTS	242.26±28.31	
			Pulp			CUPRAC	2.04±0.32	
						DPPH	0.39±0.10	
						ABTS	0.91±0.12	

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review (cont.)

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Antioxidant	Methanol	Hass	Seed	- TPC - Flavonols - Procyanidins - Catechins - OH-B - OH-C	N/A	CUPRAC	141.67±41.24	(Rodríguez-Carpena et al., 2011)
						DPPH	66.24±24.84	
						ABTS	78.93±26.73	
			Peel			CUPRAC	145.98±69.25	
						DPPH	71.92±28.93	
						ABTS	74.06±23.17	
			Pulp			CUPRAC	1.33±0.43	
						DPPH	0.32±0.07	
						ABTS	0.94±0.23	
		Fuerte	Seed			CUPRAC	184.42±66.05	
						DPPH	94.27±30.47	
						ABTS	121.61±31.87	
			Peel			CUPRAC	330.75±62.57	
						DPPH	174.71±29.80	
						ABTS	185.87±26.91	
			Pulp			CUPRAC	1.64±0.44	
						DPPH	0.29±0.09	
						ABTS	0.78±0.17	

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review (cont.)

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Antioxidant	N/A	Hass	Seed			Disk diffusion assay	7.18-9.73	(Rodríguez-Carpena et al., 2011)
			Peel				4.95-6.20	
			Pulp				5.21-9.48	
		Fuerte	Seed				5.00-9.81	
			Peel				5.11-6.91	
			Pulp				5.11-13.00	
	Aqueous + Ultrasound-assisted batch extraction (UABE)	N/A	Seed	TPC	N/A	ORAC assay	Values presented in chart, no specific values stated	(Oryan et al., 2015)
	Aqueous + Ultrasound-assisted continuous extraction (UACE)							
	Methanol	N/A	Seed	TPC including: - Tannins - Flavonoids - Alkaloids - Polyphenols Not mentioned to be correlated to antioxidant: - Anthocyanins - Anthraquinones - Triterpenes - Steroids - Saponins	N/A	Antibacterial assay	64-128	(Ekom & Kuete, 2022)
							512->2048	
						DPPH assay	55.91 ± 2.12	
						FRAP assay	N/A	

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review (cont.)

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Antioxidant	80% Methanol	Hass	Peel	Seed: - 3-O-caffeoylquinic acid - 3-O-p-coumaroylquinic acid - Procyanidins - Catechin/epicatechins	N/A	ORAC assay	0.47 ± 0.036	(Kosińska et al., 2012)
Antimicrobial	Extracted twice with ethanol at 50°C for 1 h, followed by acetone extraction at 4°C overnight. Extract was dried and re-dissolved in 35 ml hexane and refrigerated at 4°C overnight prior to filtration.	Shepard	Seed Peel	Peel: - Quercetin - Glycosides - 5-O-caffeoylquinic acid - Catechins - Procyanidins	N/A	DPPH assay ORAC assay	0.358 0.21 ± 0.014	(Rosenblat et al., 2011)
						TEAC assay DPPH assay	0.094 ± 0.0007 0.920	
						ORAC assay	0.29 ± 0.020	
						TEAC assay	0.112 ± 0.0034	
						DPPH assay	0.927	

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review (cont.)

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Anti-ageing			Seed			ORAC assay TEAC assay DPPH assay	0.35 ± 0.021	(Ferreira da Vinha et al., 2013)
			Peel				0.091 ± 0.0047	
			Seed				0.776	
	Exracted with Hexane for 14 hours, cool crystallization and filtration	N/A	Seed	N/A	-1-acetoxy-2,4-dihydroxy-heptadec-16-ene - 1-acetoxy-2,4-dihydroxy-heptadec-16-yne	Keratinocyte survival assay	91.0 ± 10	(Rosenblat et al., 2011)
	Extracted twice with ethanol at 50°C for 1 h, followed by acetone extraction at 4°C overnight. Extract was dried and re-dissolved in 35 ml hexane and refrigerated at 4°C overnight prior to filtration.		Pulp			Measurement of amount of CPD in cellular DNA post UVB irradiation	37.5 ± 6.2	
							74.5 ± 41	
						ELISA assay	50	
							>5 μg/ml	
	Methanol, Hexane	Hass	Oil	Major phenolic compounds: - P-vanillin - Quercetin - Hydroxyphenylacetic acid - α-Tocopherol	N/A	DPPH assay	N/A	(Ferreira da Vinha et al., 2013)

Table 1: Summary of extraction of bioactive compounds and its cosmeceutical benefits of studies appraised in this review (cont.)

Properties	Extract	Cultivar	Part of Avocado	Active bioactive(s)		Assay(s)/ Test(s)	Values	Reference(s)
				Extract	Pure Compound(s)			
Anti-ageing	Methanol/Chloroform, Acetone	Margarida	Pulp	Phospholipids: - PE - PC - LPC - PI	N/A	Not conducted	N/A	(Züge et al., 2017)
	Acetone, Diethyl ether, Methanol	Maluma	Pulp	- TPC - Carotenoids - Chlorophyll	N/A	- ABTS - DPPH - FRAP	N/A	(Lister et al., 2021)
Anti-tyrosinase	Acetone Diethyl ether					Agar Well Diffusion Assay	5-17	(Hürkul et al., 2021)
						N/A	0-15	

study by Melgar and colleagues (2018) showed contradictory results; the analysis using four different assays, DPPH assay, reducing power, β-carotene bleaching inhibition, TBARS assay indicated that Hass avocado peels generate lower EC50 values compared to Hass avocado seeds. Lower EC50 values indicate greater potency of antioxidant compounds.

An *in-vitro* antimicrobial activity range of 5.00-13.00 mm is yielded via disk diffusion assay with avocado seed and pulp extract (Rodríguez-Carpena et al., 2011). Avocado pulp extracted with acetone, ether and methanol via agar well diffusion assay yielded an inhibitory diameter (mm) with a range of 0-28 (Nguyen et al., 2021). Avocado pulp slices which were dried, grounded and extracted in a water-alcoholic solution showed that 80% of the final product contained mannoheptulose and perseitol, which presents the antimicrobial activity of avocado via invasion assay (Donnarumma et al., 2007; Paoletti et al., 2010).

Anti-inflammatory

Methanol extract exhibits different anti-inflammatory activities with different parts of the avocado used via HRBC membrane stabilization assay (Table 2). Avocado seed, peel and pulp yielded an IC50 (mg/mL) value of 2.03±0.06, 2.01±0.06 and 2.22±0.15. Furthermore, N-hexane exhibits different anti-inflammatory activities with different parts of the avocado used via HRBC membrane stabilization assay. Avocado seed, peel and pulp yielded an IC50 (mg/mL) value of 7.73±0.09, 9.96±1.03 and 5.89±0.89 accordingly (Hürkul et al., 2021). Avocado pulp was extracted twice with ethanol (50°C for 1 h), followed by acetone extraction at 4°C overnight, and re-desolvation in 35 ml hexane and refrigerated using ELISA assay generated a secretion value of IL-6 (PFA: 0.5 μg/mL) of 50 mg/mL (Rosenblat et al., 2011).

Avocado also showed anti-inflammatory activity when *in-vivo*. The leaves of *Persea americana* Mill. (1%, 3% and 10%) yielded the effect of anti-inflammatory when used once daily for six days on adult male Swiss mice (Deuschle et al., 2019). The oil from avocado and soybean extracts cream showed an anti-inflammatory effect when consumed twice daily for 24 weeks. The avocado used as topical 5-alpha avocuta 2% creams also yielded an anti-inflammatory effect when used on human volunteers twice daily for 14 weeks. Furthermore, for eight weeks, rats fed on 10% (w/w) of avocado oils (C-RAO-C, E-URAO-I) also showed an anti-inflammatory effect.

The methanol extract and N-hexane of the avocado seed, peel, and pulp all yield-ed different IC50 values, suggesting that the anti-inflammatory activity may vary de-pending on the part of the plant used. The *in-vivo* studies also show promising results, with the leaves of *Persea americana* Mill. and avocado showing anti-inflammatory effects on mice and humans, respectively. The use of avocado oil in rats also yielded positive results. It would be worthwhile to conduct further research to investigate avocados' potential as an anti-inflammatory agent and determine the specific compounds responsible for these activities.

Wound healing

Avocado has shown significant benefits in wound healing. A study by Oryan and colleagues (2015), showed that an amount of 10mg/ml of Avocado/Soybean Unsaponifiable (ASU) applied to the wound area significantly increased collagen levels in comparison to control groups (P=0.001). Another study by Lamaud and colleagues (1982) on the production of collagen using programmed differential calorimetry reported that avocado and soya bean lipidic non-saponifiables (PIAS) reported a high score for thickness (1.25 ±0.02 N mm-2), elasticity (18.9±0.40 N mm-2), resistance to rupture (8.1±0.25 N mm-2) and elongation to rupture (60.9±1.07%) indicating a high collagen production. Hence, it is shown that avocados can increase the elasticity and reduce the size of the wound in a shorter period of time and in the zone of application, the thermal stability of collagen decreases.

Furthermore, oleic acid, also known as omega-9 fatty acid, was reported as the most prominent active ingredient, with the highest content of 47.20% (Sichani et al., 2021). Interestingly, oleic acid was

also found in omega-50% SSFAO or avocado oil, which is used to apply on the wound. Studies have reported that it helps with the contraction of the wound by increasing the anti-inflammatory action with 50% SSFAO or in natural avocado oil (2.50 ± 0.15 cells, 2.71 ± 0.12 cells) in comparison with the EFA control (10.00 ± 0.41 cells) and petroleum jelly control (28.82 ± 1.70 cells). This could be due to the beneficial properties of the avocado such as polyunsaturated fatty acids (PUFA), mono-unsaturated fatty acids (MUFA), beta-sitosterol, beta-carotene, lecithin, minerals, vitamin A, C, D, E, linoleic acid and linolenic acid that allows better wound healing (Sichani et al., 2021).

Anti-tyrosinase

Tyrosinase is an enzyme that initiates the production of melanin, resulting in a darker skin tone (Hürkul et al., 2021). However, constant exposure to UV lights will instantly activate the production of melanin, protecting the skin and thus causing a darker skin tone (Alves et al., 2019). Several studies have investigated the potential of avocado bioactive compounds as skin whitening agents, particularly due to their ability to inhibit the activity of tyrosinase, the enzyme responsible for melanin production.

Catechins structure is reported to enhance the inhibition of tyrosinase activity and in the avocado, it is reported as an effective skin-whitening agent (Laksmiani et al., 2020). The ethyl acetate extract of avocado seeds (25.5%) showed the most catechins compared to ethanol extract (20.87%) and acetone extract (14.48%). The optimal extract that can be used in dermatological applications is n-hexane extracts on exocarp and seeds.

Laksmiani and colleagues (2020) found that avocado fruit extract could effectively inhibit tyrosinase activity and reduce melanin production in human melanoma cells. This study suggests that avocado fruit extract could be used as a natural skin-whitening agent. Another study by Nazir and colleagues (2018) investigated the skin-whitening potential of avocado seed extracts. The study found that the ethyl acetate extract of avocado seeds had the highest concentration of catechins, which are known to inhibit tyrosinase activity. The n-hexane extract of avocado seeds also had skin-whitening properties.

In addition to inhibiting tyrosinase activity, avocado bioactive compounds also protect the skin from UV-induced damage, which can cause hyperpigmentation. The antioxidant properties of avocado, particularly the presence of carotenoids and poly-phenols, can provide photoprotective effects and prevent skin damage caused by UV radiation.

Anti-aging

Anti-ageing studies have also shown that Avocado contains essential fatty acids such as linoleic acid, oleic acid, and linolenic acid, which contribute to hydration, elasticity and firmness of the skin. These properties play major roles in preventing and reducing wrinkles and fine lines. The study by Putri and colleagues (2018) focused on the anti-oxidant capacity of avocado oil topical application, showing that topical application of avocado oil results in smoother skin texture (Putri et al., 2018). Furthermore, Avocado unsaponifiable can be incorporated into topical creams and has been found to be effective as an alternative in treating dermatological conditions such as vulvar lichen sclerosus (VLS) (Felmingham et al., 2020).

Polyhydroxylated fatty alcohols (PFA), specifically 1-acetoxy-2,4-dihydroxy-heptadec-16-ene (Kosińska et al., 2012) and 1-acetoxy-2,4-dihydroxy-heptadec-16-yne (Ferreira da Vinha et al., 2013) have been extracted from avocado seed and pulp with hexane, ethanol and acetone. These two compounds exhibited photo-protective properties, which can slow down the process of skin ageing. The cell viability of keratinocytes treated with PFA of 0.5 μg/ml was 91.0 ± 10% after exposure to UVB irradiation at a dose of 20 mJ/cm (Table 1). The cell viability of PFA-treated samples was much higher than non-PFA-treated samples, with cell viability of 61.0 ± 3%, suggesting that PFA reduces UVB-induced cell death. PFA was also reported to enhance DNA repair in UVB-irradiated keratinocytes (Rosenblat et al., 2011). In another study, Mwinga and colleagues (2019) showed that

avocado leaves and pulp had been used topically for anti-ageing purposes such as improving skin complexion, sunlight protection, removing pigmentation and making the skin soft- over the years.

Avocado oil is rich in essential fatty acids such as linoleic acid, oleic acid, palmitic acid and omega fatty acids such as triglycerides and phytosterol. These compounds contribute to skin moisturization by restoring the hydro-lipid shielding skin barrier (Naeimifar et al., 2020). A study on topical cream application with 2% avocado oil twice daily for 4 weeks showed an increase in hydration and an improvement in skin barrier function leading to better skin appearance (Moldovan et al., 2021). Another study was carried out for 12 weeks and reported that wrinkles were reduced from 9.50 ± 1.19 (baseline) to 9.35 ± 1.42 and the volume of nasolabial folds dropped (from 4.68 ± 1.83 (baseline) to 4.33 ± 1.77). These properties can be attributed to polyphenols, triglycerides, proteins, and vitamins A, D, and E (Naeimifar et al., 2020).

In the study by Lister and colleagues (2021), ointments containing different avocado peel extracts were applied twice daily for four weeks on rat skins, which showed higher avocado concentrations of peel extract lead to a higher percentage of hydration, elasticity and collagen levels. Oral consumption of an avocado has also been proven to increase the firmness and elasticity of the skin (Susanne M Henning et al., 2022).

Another study by Naeimifar and colleagues (2020) investigated the effect of avocado oil on skin hydration and elasticity. The research revealed that applying avocado oil topically can notably enhance skin hydration and boost skin elasticity, suggesting its potential as an ingredient in anti-aging products. Moreover, regular consumption of avocado may result in improved elasticity and firmness of facial skin among healthy women (Henning et al., 2022).

Moreover, the phytosterols found in avocado, such as beta-sitosterol, have been shown to have anti-aging properties. These compounds can improve skin texture, reduce the appearance of age spots, and promote collagen production, which can lead to firmer and more youthful-looking skin.

The polyhydroxylated fatty alcohols (PFA) found in avocado seed and pulp showed photo-protective properties; enhancing DNA repair in UVB-irradiated keratinocytes, which can prevent skin damage and premature ageing. The essential fatty acids and phytosterols in avocado oil contribute to skin moisturization and improve skin barrier function, leading to better skin appearance. The consumption of avocados can enhance the firmness and elasticity of the skin.

Bioactive compound extraction methods

Table 1 also lists various extraction techniques used on different parts of the avocado (*Persea americana* Mill), highlighting the resulting bioactive compounds and their potential benefits in cosmetics. Ethyl acetate extracts from the seeds, peel, and pulp of Hass and Fuerte cultivars contain bioactive such as TPC, flavanols, procyanidins, catechins, OH-B, and OH-C, which exhibit strong antioxidant properties (Rodríguez-Carpena et al., 2011). These antioxidants can reduce oxidative stress and mitigate signs of ageing. Similarly, acetone extracts from these parts are rich in antioxidants, further enhancing their anti-ageing potential. Methanol extracts, particularly from the seeds of both cultivars, yield significant quantities of tannins, flavonoids, alkaloids, and polyphenols, all of which contribute to skin protection and rejuvenation (Ekom & Kuete, 2022). Additionally, aqueous and methanol extracts, including ultrasound-assisted techniques, effectively extract high levels of polyphenols and other phytochemicals, which offer broad-spectrum antimicrobial and anti-inflammatory benefits essential for wound healing and skin health. Thus, the extraction technique and avocado part significantly influence the presence and concentration of bioactive compounds, determining their specific cosmetic applications.

Acetone extracts have been found to exhibit the greatest antioxidant capacity, followed by methanol and ethyl acetate, and the ratio of acetone has also been shown to affect the antioxidant capacity of avocado extracts. Overall, the varying levels of antioxidant capacity exhibited by different avocado cultivars and plant parts highlight the importance of

an appropriate extraction method and solvent.

Methanol extracts produce the highest MIC (μg/mL) value presented by its MIC (μg/mL) of 64-128 and MBC (μg/mL) of 512->2048 via antimicrobial assay when extracting avocado seed (Lin et al., 2017). Whereas avocado peel extracted with 80% ethanol via antibacterial assay produces a MIC (mg/ml) of 0.015-0.030 and MBC (mg/mL) of 0.030-0.450. Furthermore, when the avocado seed was extracted with 80% ethanol via antibacterial assay, a MIC (mg/mL) of 0.020-0.150 and MBC (mg/mL) of 0.030-0.300 was detected. Whereas extraction via antifungal assay showed a MIC (mg/mL) of 0.020-0.300.

In-vitro and in-vivo studies on bioactive compounds of avocado

In-vivo studies revealed that the application of bio-active compounds from avocados accelerates the wound-healing process by promoting epithelialization and wound contraction, while also reducing inflammatory cells (Table 2).

Table 2 summarizes a comparison of the antioxidant properties of avocado extracts from different cultivars, using various solvents and extraction techniques. Ethyl acetate extraction demonstrated moderate antioxidant activity in CUPRAC assays (seeds: 58.00 ± 15.55, peels: 56.40 ± 21.19), but relatively lower activity in DPPH assays (seeds: 17.78 ± 4.34, peels: 17.85 ± 7.07) and ABTS assays (seeds: 21.57 ± 7.51, peels: 16.12 ± 6.98). This method effectively extracts phenolic compounds such as flavonols, procyanidins, and catechins, but results in lower antioxidant activities compared to other solvents due to limited solubility of specific bioactive compounds.

On the other hand, acetone extraction demonstrated significantly higher antioxidant activities across all assays, particularly in the CUPRAC (Hass seed: 275.36 ± 59.09, peel: 218.04 ± 42.42; Fuerte seed: 353.43 ± 75.83, peel: 456.24 ± 77.07) and DPPH assays (Hass seed: 130.26 ± 36.80). The high efficacy of acetone extraction can be attributed to its ability to dissolve a wider range of antioxidant compounds. However, it may also extract non-target substances, which complicates the extraction process.

Each extraction method has its advantages and disadvantages, with ethyl acetate offering specificity for certain phenolics, and acetone providing higher overall antioxidant yields, but with the potential for extracting a broader range of compounds.

In-vivo studies suggest that avocado bioactive compounds may aid in wound healing and have anti-aging effects on the skin (Susanne M. Henning et al., 2022)

The inclusion of *in vivo* and *in vitro* studies provides a more comprehensive understanding of the potential benefits of these compounds, however, there are still several limitations to consider. One limitation is the wide variety of sample sizes and study designs of studies included in this review; making it difficult to compare and generalize the results, as different sample sizes and types may produce different out-comes. The use of animal models in vivo studies may not accurately reflect the out-comes of cosmeceutical actions on human skin due to differences in skin structures. Another limitation is excluding studies published in languages other than English, which may result in selection bias, potentially limiting the scope of the review, as important studies published in other languages may have been missed.

Conclusion

To conclude, review summarizes the antioxidant, antimicrobial, anti-inflammatory, wound healing, anti-ageing and anti-tyrosinase properties activities of *Persea americana* Mill. and its potential application in cosmeceuticals. It is clear that there is growing interest in the potential benefits of avocado bioactive compounds, including their antioxidant, antimicrobial, anti-inflammatory, wound healing, anti-aging, and anti-tyrosinase properties. The review also highlights the importance of considering the different bioactive compound extraction solvents and methods used, as this can have a significant impact on the effectiveness of these compounds. However, further research is needed to understand better the mechanisms by which these compounds act on the skin and to optimize extraction methods to improve the yield.

Table 2: *In-vivo* studies on cosmeceutical activities of avocado

Cosmeceutical Action	Mechanism of action	Compound used	Part of avocado	Amount	Duration	Sample	Compound	References
Wound healing	Increase rate of wound contraction and epithelialization	Avocado/soybean unsaponifiables (ASU) topical cream with a ratio of 1:2.	Avocado unsaponifiables	1mL ASU/cream (10mg ASU/ml cream)	10 days	Adult male Wistar rats	- Polyunsaturated fatty acids (PUFA) - Monounsaturated fatty acids (MUFA) - β-sitosterol - β-carotene - Lecithin - Minerals - Vitamins A, C, D, and E - Oleic acid - Linoleic acid - Linolenic acid	(Oryan et al., 2015)
	Promote increased collagen synthesis and decreased numbers of inflammatory cells	Semisolid formulation of Avocado Oil (SSFAO 50%)	NA	±100 mg once daily	14 days	64 adult Wistar rats, male and female, with ages between 3-4 months and weighing 200–250g	- Flavonoids - Polyphenols - Tannins	(A. P. de Oliveira et al., 2013)
	- Increase the rate of wound contraction and epithelialization - Reduce the number of Colony Forming Units (CFU) of S. aureus at the infection site	Carbopol (1%) gels containing 1%, 5% and 10% MeOH extract of P. americana.	Seed	Once daily	20 days	63 male Wistar albino rats aged 8-10 weeks (150–200g)	- Unsaturated fatty acids (PUFAs) - Linoleic acid - Linolenic acids - Unsaturated fatty acids (MUFAs) - Oleic acid - Beta-sitosterol - Beta-carotene - Lecithin - Minerals - Vitamins A, C, D, and E. - Fatty acids (oleic, linoleic, and linolenic acids	(Ekom & Kuete, 2022)

Table 2: *In-vivo* studies on cosmeceutical activities of avocado (cont.)

Cosmeceutical Action	Mechanism of action	Compound used	Part of avocado	Amount	Duration	Sample	Compound	References
Wound healing	Increase collagen synthesis, reduce the number of inflammatory cells, accelerate the process of coagulation and accelerate the regeneration of epithelium thus help increase the wound healing process	Avocado Oil derived from squeezing avocado paste (Iran)	Oil	Twice daily	14 days	30 Winstar Rats (divided into 3 groups, 10 each)	- Fatty essentials acids like linoleic (6 - 30%), linolenic (0.4 - 4%) acids, and oleic acid (31 - 70%) - β-sitosterol - β-carotene - Lecithin - Minerals - Vitamins A, C, D and E	(Sichani et al., 2021)
Anti-aging	- Repair dry, damaged or chapped skin - Increase hydration - Improve or restore skin barrier function - Improve skin appearance	O/W and W/O creams containing 2% of avocado oil	Oil	Twice a day	4 weeks	O/W - 4 volunteers (23-50 years old) W/O - Volunteers (35-55 years old)	- Oleic acid - Linoleic acid - Palmitic acid - Phytosterols - Polyphenols - Triglycerides - Proteins - Vitamin A, D, E	(Moldovan et al., 2021)
	- Promotes firmness and elasticity that helps to reduce wrinkles without altering the hydration index	Topical Cream (O/W) - Avocado Oil, Saffron extract and honey fragrance obtained from Barij Essence Company	Oil	Apply ONE fingertip unit of cream on the face once daily	12 weeks	20 participants of both genders	- Alkaloids - Tannins - Phenols - Flavonoids - Glycosides	(Naeimifar et al., 2020)
	Increase average hydration, elasticity, and collagen levels of skin	Avocado Peel Extract Ointment 2.5%, 5%, 7.5%, 10%	Peel	Twice daily	4 weeks	25 male rats	- Lutein - Zeaxanthin	(Lister et al., 2021)

Table 2: *In-vivo* studies on cosmeceutical activities of avocado (cont.)

Cosmeceutical Action	Mechanism of action	Compound used	Part of avocado	Amount	Duration	Sample	Compound	References
Anti-aging	Increased firmness and elasticity of the forehead and reduced tiring of repeat stretching of the forehead skin	*Persea Americana* oral consumption	Fruit	One avocado once daily	8 weeks	39 female participants with Fitzpatrick skin type II-IV	- Phenolic compounds such as caffeic, ferulic, vanillic, and hydroxybenzoic acids and flavonoids such as quercetin, and kaempferol - Catechin - Chlorogenic acid - Rutin	(Susanne M Henning et al., 2022)
Anti-inflammatory	- Prevent UVB-induced mechanical allodynia - Antinociceptive - Anti-inflammatory	*Persea Americana* (1%, 3%, 10%)	Leaves	Once daily	6 days	Adult male Swiss mice (25–30 g)	ASE cream: - Hyaluronic acid - Vitamin E - Sodium carboxymethyl beta glucan - dimethylmethoxy chromanol - trimethylglycine ASE supplement: - Vitamin E - Para-aminobenzoic acid (PABA) - Phytosterols	(Deuschle et al., 2019)
	- Anti-fibrotic, emollient, lenitive actions - Effective alternatives in the treatment of symptoms and signs of mild to moderate vulvar lichen sclerosus (VLS)	Avocado and soybean extracts (ASE) cream and dietary supplements	Oil	Twice daily	24 weeks; 12 weeks	23 participants	Five-Alpha Avocuta	(Borghi et al., 2015)

Authors contributions

Conceptualization, G.B.H; methodology, S.M; formal analysis, Y.L.L, L.X.N., L.W.Y.; data curation, Y.L.L, L.X.N., L.W.Y writing—original draft preparation, Y.L.L, L.X.N., L.W.Y.; writing—review and editing, S.M., G.B.H.; supervision, G.B.H. All authors have read and agreed to the published version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

References

Almanasef, M. (2021). Mental health literacy and help-seeking behaviours among undergraduate pharmacy students in abha, saudi arabia. Risk Management and Healthcare Policy, 14, 1281–1286. https://doi.org/10.2147/RMHP.S289211

Alves, A. Q., da Silva Jr, V. A., Góes, A. J. S., Silva, M. S., de Oliveira, G. G., Bastos, I. V. G. A., de Castro Neto, A. G., & Alves, A. J. (2019). The fatty acid composition of vegetable oils and their potential use in wound care. *Advances in skin & wound care*, *32*(8), 1-8.

Borghi, A., Corazza, M., Minghetti, S., Toni, G., & Virgili, A. (2015). Avocado and soybean extracts as active principles in the treatment of mild-to-moderate vulvar lichen sclerosus: results of efficacy and tolerability. *Journal of the European Academy of Dermatology and Venereology*, *29*(6), 1225-1230.

de Oliveira, A. P., Franco Ede, S., Rodrigues Barreto, R., Cordeiro, D. P., de Melo, R. G., de Aquino, C. M., AA, E. S., de Medeiros, P. L., da Silva, T. G., Góes, A. J., & Maia, M. B. (2013). Effect of semisolid formulation of ***Persea americana*** Mill (avocado) oil on wound healing in rats. *Evid Based Complement Alternat Med*, *2013*, 472382. https://doi.org/10.1155/2013/472382

de Oliveira, A. P., Franco, E. d. S., Rodrigues Barreto, R., Cordeiro, D. P., de Melo, R. G., de Aquino, C. M. F., e Silva, A. A. R., de Medeiros, P. L., da Silva, T. G., Góes, A. J. d. S., & Maia, M. B. d. S. (2013). Effect of Semisolid Formulation of *Persea americana* Mill (Avocado) Oil on Wound Healing in Rats. *Evidence-Based Complementary and Alternative Medicine*, *2013*, 472382. https://doi.org/10.1155/2013/472382

Deuschle, V. C., Brusco, I., Piana, M., Faccin, H., de Carvalho, L. M., Oliveira, S. M., & Viana, C. (2019). *Persea americana* Mill. crude extract exhibits antinociceptive effect on UVB radiation-induced skin injury in mice. *Inflammopharmacology*, *27*(2), 323-338.

Donnarumma, G., Buommino, E., Baroni, A., Auricchio, L., Filippis, A. D., Cozza, V., Msika, P., Piccardi, N., & Tufano, M. A. (2007). Effects of AV119, a natural sugar from avocado, on Malassezia furfur invasiveness and on the expression of HBD-2 and cytokines in human keratinocytes. *Experimental dermatology*, *16*(11), 912-919.

Dreher, M. L., & Davenport, A. J. (2013). Hass avocado composition and potential health effects. *Crit Rev Food Sci Nutr*, *53*(7), 738-750. https://doi.org/10.1080/10408398.2011.556759

Ekom, S. E., & Kuete, V. (2022). Methanol extract from the seeds of *Persea americana* displays antibacterial and wound healing activities in rat model. *Journal of Ethnopharmacology*, *282*, 114573.

Felmingham, C., Chan, L., Doyle, L. W., & Veysey, E. (2020). The Vulval Disease Quality of Life Index in women with vulval lichen sclerosus correlates with clinician and symptom scores. *Australasian Journal of Dermatology*, *61*(2), 110-118. https://doi.org/https://doi.org/10.1111/ajd.13197

Ferreira da Vinha, A., Moreira, J., & Barreira, S. (2013). Physicochemical parameters, phytochemical composition and antioxidant activity of the algarvian avocado (*Persea americana* Mill.). *Journal of Agricultural Science*, *5*(12), 100-109.

Forero-Doria, O., García, M. F., Vergara, C. E., & Guzman, L. (2017). Thermal analysis and antioxidant activity of oil extracted from pulp of ripe avocados. *Journal of Thermal Analysis and Calorimetry*, *130*(2), 959-966.

García-Villegas, A., Rojas-García, A., Villegas-Aguilar, M. D. C., Fernández-Moreno, P., Fernández-Ochoa, Á., Cádiz-Gurrea, M. d. l. L., Arráez-Román, D., & Segura-Carretero, A. (2022). Cosmeceutical Potential of Major Tropical and Subtropical Fruit By-Products for a Sustainable Revalorization. *Antioxidants (Basel, Switzerland)*, *11*(2), 203. Retrieved 2022/01//, from http://europepmc.org/abstract/MED/35204085

García-Villegas A, Rojas-García A, Villegas-Aguilar MDC, et al. Cosmeceutical Potential of Major Tropical and Subtropical Fruit By-Products for a Sustainable Revalorization. Antioxidants (Basel, Switzerland). 2022 Jan;11(2):203. DOI: 10.3390/antiox11020203. PMID: 35204085; PMCID: PMC8868306.

Henning, S. M., Guzman, J. B., Thames, G., Yang, J., Tseng, C.-H., Heber, D., Kim, J., & Li, Z. (2022). Avocado Consumption Increased Skin Elasticity and Firmness in Women - A Pilot Study. *Journal of cosmetic dermatology*, *21*(9), 4028-4034. https://doi.org/https://doi.org/10.1111/jocd.14717

Henning, S. M., Guzman, J. B., Thames, G., Yang, J., Tseng, C. H., Heber, D., Kim, J., & Li, Z. (2022). Avocado Consumption Increased Skin Elasticity and Firmness in Women-A Pilot Study. *Journal of cosmetic dermatology*.

Hürkul, M.-M., Sarıaltın, S.-Y., Köroğlu, A., & Çoban, T. (2021). In vitro inhibitory potential of avocado fruits, *Persea americana* (Lauraceae) against oxidation, inflammation and key enzymes linked to skin diseases. *Revista de Biología Tropical*, *69*(2), 472-481.

Husein El Hadmed, H., & Castillo, R. F. (2016). Cosmeceuticals: peptides, proteins, and growth factors. *J Cosmet Dermatol*, *15*(4), 514-519. https://doi.org/10.1111/jocd.12229

Kosińska, A., Karamać, M., Estrella, I., Hernández, T., Bartolomé, B., & Dykes, G. A. (2012). Phenolic compound profiles and antioxidant capacity of *Persea americana* Mill. peels and seeds of two varieties. *Journal of agricultural and food chemistry*, *60*(18), 4613-4619.

Lagoa, R., Silva, J., Rodrigues, J. R., & Bishayee, A. (2020). Advances in phytochemical delivery systems for improved anticancer activity. *Biotechnology advances*, *38*, 107382.

Laksmiani, N. P. L., Sanjaya, I. K. N., & Leliqia, N. P. E. (2020). The activity of avocado (*Persea americana* Mill.) seed extract containing catechin as a skin lightening agent. *J Pharm Pharmacogn Res*, *8*, 449-456.

Lamaud, E., Huc, A., & Wepierre, J. (1982). Effects of avocado and soya bean lipidic non-saponifiables on the components of skin connective tissue after topical application in the hairless rat: biophysical and biomechanical determination. *International journal of cosmetic science*, *4*(4), 143-152.

Lin, T.-K., Zhong, L., & Santiago, J. L. (2017). Anti-inflammatory and skin barrier repair effects of topical application of some plant oils. *International journal of molecular sciences*, *19*(1), 70.

Lister, I., Amiruddin, H. L., Fachrial, E., & Girsang, E. (2021). Anti-Aging Effectiveness of Avocado Peel Extract Ointment (*Persea americana* Mill.) against Hydration, Collagen, and Elasticity Levels in Wistar Rat. *Journal of Pharmaceutical Research International*, 173-184.

Melgar, B., Dias, M. I., Ciric, A., Sokovic, M., Garcia-Castello, E. M., Rodriguez-Lopez, A. D., Barros, L., & Ferreira, I. C. (2018). Bioactive characterization of *Persea americana* Mill. by-products: A rich source of inherent antioxidants. *Industrial Crops and Products*, *111*, 212-218.

Moldovan, M. L., Ionuț, I., & Bogdan, C. (2021). Cosmetic products containing natural based emollients for restoring impaired skin barrier: formulation and in vivo evaluation. *Farmacia*, *69*(1), 129-134.

Mwinga, J., Makhaga, N., Aremu, A., & Otang-Mbeng, W. (2019). Botanicals used for cosmetic purposes by Xhosa women in the Eastern Cape, South Africa. *South African Journal of Botany*, *126*, 4-10.

Naeimifar, A., Ahmad Nasrollahi, S., Samadi, A., Talari, R., Sajad Ale-nabi, S., Massoud Hossini, A., & Firooz, A. (2020). Preparation and evaluation of anti-wrinkle cream containing saffron extract and avocado oil. *Journal of cosmetic dermatology*, *19*(9), 2366-2373.

Nguyen, T.-V.-L., Nguyen, Q.-D., Nguyen, N.-N., & Nguyen, T.-T.-D. (2021). Comparison of Phytochemical Contents, Antioxidant and Antibacterial Activities of Various Solvent Extracts Obtained from 'Maluma'Avocado Pulp Powder. *Molecules*, *26*(24), 7693.

Nilforoushzadeh, M. A., Amirkhani, M. A., Zarrintaj, P., Salehi Moghaddam, A., Mehrabi, T., Alavi, S., & Mollapour Sisakht, M. (2018). Skin care and rejuvenation by cosmeceutical facial mask. *Journal of cosmetic dermatology*, *17*(5), 693-702.

Oryan, A., Mohammadalipour, A., Moshiri, A., & Tabandeh, M. R. (2015). Avocado/soybean unsaponifiables: a novel regulator of cutaneous wound healing, modelling and remodelling. *International Wound Journal*, *12*(6), 674-685.

Pandey, H., & Kumar, S. (2021). Butylated hydroxytoluene and Butylated hydroxyanisole induced cyto-genotoxicity in root cells of Allium cepa L. *Heliyon*, *7*(5), e07055. https://doi.org/10.1016/j.heliyon.2021.e07055

Paoletti, I., Buommino, E., Tudisco, L., Baudouin, C., Msika, P., Tufano, M. A., Baroni, A., & Donnarumma, G. (2010). Patented natural avocado sugars modulate the HBD-2 expression in human keratinocytes through the involvement of protein kinase C and protein tyrosine kinases. *archives of dermatological research*, *302*(3), 201-209.

Poljšak, N., Kreft, S., & Kočevar Glavač, N. (2020). Vegetable butters and oils in skin wound healing: Scientific evidence for new opportunities in dermatology. *Phytother Res*, *34*(2), 254-269. https://doi.org/10.1002/ptr.6524

Puglia, C., & Santonocito, D. (2019). Cosmeceuticals: nanotechnology-based strategies for the delivery of phytocompounds. *Current pharmaceutical design*, *25*(21), 2314-2322.

Putri, T., Raya, I., Natsir, H., & Mayasari, E. (2018). Chlorella sp: Extraction of fatty acid by using avocado oil as solvent and its application as an anti-aging cream. Journal of Physics: Conference Series,

Rafique, S., & Akhtar, N. (2018). Phytochemical analysis and antioxidant activity of Persia americana and Actinidia deliciosa fruit extracts by DPPH method. *Biomed. Res*, *29*(12), 2459-2464.

Rodríguez-Carpena, J.-G., Morcuende, D., Andrade, M.-J., Kylli, P., & Estévez, M. (2011). Avocado (*Persea americana* Mill.) phenolics, in vitro antioxidant and antimicrobial activities, and inhibition of lipid and protein oxidation in porcine patties. *Journal of agricultural and food chemistry*, *59*(10), 5625-5635.

Romes, N. B., Abdul Wahab, R., & Abdul Hamid, M. (2021). The role of bioactive phytoconstituents-loaded nanoemulsions for skin improvement: a review. *Biotechnology & Biotechnological Equipment*, *35*(1), 711-730. https://doi.org/10.1080/13102818.2021.1915869

Rosenblat, G., Meretski, S., Segal, J., Tarshis, M., Schroeder, A., Zanin-Zhorov, A., Lion, G., Ingber, A., & Hochberg, M. (2011). Polyhydroxylated fatty alcohols derived from avocado suppress inflammatory

response and provide non-sunscreen protection against UV-induced damage in skin cells. *archives of dermatological research*, *303*(4), 239-246.

Samtiya, M., Aluko, R. E., Dhewa, T., & Moreno-Rojas, J. M. (2021). Potential Health Benefits of Plant Food-Derived Bioactive Components: An Overview. *Foods*, *10*(4). https://doi.org/10.3390/foods10040839

Sharquie, K. E., Al-Hamamy, H. R., Noaimi, A. A., & Tahir, A. F. (2012). Treatment of Acne Vulgaris with 5-Alpha Avocuta Cream 2% in Comparison with Tretinion Cream 0.025%(Single Blind Comparative Study). *Journal of Cosmetics, Dermatological Sciences and Applications*, *2*(3), 179.

Sichani, M. R. E., Farid, M., & Khorasgani, E. M. (2021). Histomorphological Examination of Skin Wound Healing Under the Effect of Avocado Oil in Wistar Rats. *Acta Veterinaria Eurasia*, *47*(3), 121-129.

Smit, N., Vicanova, J., & Pavel, S. (2009). The hunt for natural skin whitening agents. *International journal of molecular sciences*, *10*(12), 5326-5349.

Thiyagarasaiyar, K., Goh, B.-H., Jeon, Y.-J., & Yow, Y.-Y. (2020). Algae metabolites in cosmeceutical: An overview of current applications and challenges. *Marine drugs*, *18*(6), 323.

Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K. K., Colquhoun, H., Levac, D., Moher, D., Peters, M. D. J., Horsley, T., Weeks, L., Hempel, S., Akl, E. A., Chang, C., McGowan, J., Stewart, L., Hartling, L., Aldcroft, A., Wilson, M. G., Garritty, C., . . . Straus, S. E. (2018). PRISMA Extension for Scoping Reviews (PRISMA-ScR): Checklist and Explanation. *Ann Intern Med*, *169*(7), 467-473. https://doi.org/10.7326/m18-0850

Vinha, A. F., Barreira, S. V., Castro, A., Costa, A., & Oliveira, M. B. P. (2013). Influence of the storage conditions on the physicochemical properties, antioxidant activity and microbial flora of different tomato (Lycopersicon esculentum L.) cultivars. *Journal of Agricultural Science*, *5*(2), 118.

Wang, W., Bostic, T. R., & Gu, L. (2010). Antioxidant capacities, procyanidins and pigments in avocados of different strains and cultivars. *Food chemistry*, *122*(4), 1193-1198.

Yon, J.-A.-L., Lee, S.-K., Keng, J.-W., Chow, S.-C., Liew, K.-B., Teo, S.-S., Shaik Mossadeq, W. M., Marriott, P. J., Akowuah, G. A., Ming, L. C., Goh, B. H., & Chew, Y.-L. (2023). Cassia alata (Linnaeus) Roxburgh for Skin: Natural Remedies for Atopic Dermatitis in Asia and Their Pharmacological Activities. *Cosmetics*, *10*(1), 5. https://www.mdpi.com/2079-9284/10/1/5

Züge, L. C. B., Maieves, H. A., Silveira, J. L. M., da Silva, V. R., & de Paula Scheer, A. (2017). Use of avocado phospholipids as emulsifier. *LWT-Food Science and Technology*, *79*, 42-51.

Journal of Pharmacy

Fabrication, Applications and Future Prospects of Mesoporous Silica Nanoparticles

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Abstract

In past decades, nanomedicine has become a prominent area of focus within the discipline of nanotechnology, eliciting significant anticipation within the field of biomedical research. Scientists are creating unique nanoparticles for diagnosis, utilising techniques for imaging as well as therapy applications using medication delivery techniques. Mesoporous silica nanoparticles (MSNs), a recent addition to this area, serve as a sterling example of innovative nanostructures that offer distinctive and exceptional features. These features make them valuable for developing drug delivery systems with consistent and positive advancements in preclinical. MSNs efficiently encapsulate, control, and sometimes deliver biologic agents intracellularly for clinical use due to their distinct physicochemical characteristics, such as high porosity, large surface area, adjustable pore size and dimensions, good biocompatibility, and significant loading capacity. In this article, we discuss the latest advancements in fabrication, their presumed usefulness in delivering medications, and their application as diagnostic tools. It has been demonstrated that silica can store and release therapeutics, such as antibiotics, in a sustained and controlled manner. The desirable properties of MSNs have been further enhanced by modifying the surface of the siliceous frameworks through incorporating supramolecular assemblies and various metal species and their conjugates. These substantial advancements in innovative colloidal inorganic nanocontainers have driven researchers to explore their use in novel applications, such as stimuli (light/ultrasound/ magnetic)-responsive delivery-associated therapies with exceptional in vivo performance. This article provides a brief overview of the fabrication of siliceous frameworks and discusses significant advances in the engineering of MSNs. The precise control of the shape, dimension, homogeneity, and dispersity of MSNs is crucial, as these characteristics are critical quality attributes necessary for regulatory approval. Currently, explicit FDA guidelines for developing nanomaterial-based formulations intended for diagnostic or therapeutic purposes are lacking. Therefore, establishing standardised protocols and techniques for the synthesis and characterisation of nanoparticles, particularly for their use as theranostics, is essential for future commercial potential.

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Introduction

The emergence of nanotechnology has yielded robust methodologies for fabricating nanostructured substances with significant potential for various biomedical applications, including drug delivery, illness diagnosis, medical imaging, and tissue regeneration. Nanomaterials have been utilised as carriers for active pharmaceutical ingredients to enhance distribution and targeting in biological and medical imaging applications (Fadeel & Garcia-Bennett, 2010). Experts have shown considerable interest in developing nanocarriers for the controlled and targeted delivery and release of drugs at specific disease sites (Scicluna & Vella-Zarb, 2020).

The molecules under consideration are classified as organic or inorganic nanocarriers, which demonstrated significant success in treating several infections. These organic nanometric molecules are encompassed within various polymeric agents (Begines et al., 2020), lipid-based transporters (Plaza-Oliver et al., 2021), dendrimers (Mandal, 2021) and micelles (Atanase, 2021). In contrast, inorganic nanocarriers have garnered significant interest compared to their organic counterparts due to their superior physicochemical and thermochemical stability. The primary molecules utilised in this context predominantly encompass tiny carbon, metal, or silica carriers. Inorganic nanoparticles such as quantum dots, mesoporous silica, carbon nanotubes, and gold, silver, or iron nanoparticles have the advantages of being hydrophilic, non-toxic, and biocompatible with living systems. Furthermore, the stability of inorganic nanoparticles is superior to that of organic nanoparticles. Mesoporous Silica Nanoparticles (MSNs), compared to organic carriers, such as micelle, gel, and liposome, have higher loading capacity (Yu et al. 2018). Due to their high drug encapsulation efficiency, they significantly affect nanobiotechnology research. MSNs have been successfully used as a carrier for the oral delivery of hydrophobic drugs, such as praziquantel in murine Schistosomiasis mansoni, significantly increasing the dissolution rate and bioavailability compared to standard drugs (Tawfeek et al. 2019). Recent advancements have led to the development of MSN-based drug delivery systems for the treatment of periodontitis, cancer, dentin hypersensitivity, and dental cavities.

MSNs are inorganic molecules characterised by their nano-sized pores, typically ranging from 1 to 100 nm (Manzano & Vallet-Regí, 2020; Wu et al., 2013; Zhang et al., 2018). They have strong biocompatibility yet have a mild degradation trend. Regarding dimensions, apertures size, and shape, the aforementioned mediums shared major characteristics such as excellent loading and encapsulating performance, quick and simple manufacturing technique, biocompatibility, no early discharge, and enhanced customisation (Alyassin et al., 2020).

The most advantageous characteristics of MSNs as a medication delivery method is their capacity for "zero premature controlled release", as elucidated by Slowing et al. in 2008. This property ensures the delivery of drugs without undesired leakage. Achieving this attribute involves designing MSNs as intelligent drug delivery carriers, allowing for targeted drug release in specific areas of interest while avoiding premature release at off-target sites. However, there remains a lack of information concerning the challenges encountered in their fabrication, application, and clinical translations. Therefore, this review aims to explore these issues, with particular emphasis on the regulatory aspects associated with theirs theranostic applications.

Types of MSN

The synthetic production of MSNs can be traced back to before the 1970s (Danks et al., 2016; Stober et al., 1968). In 1992, Mobil Research and Development Corporation successfully synthesised MSNs using alumino-silicate gels (Danks et al., 2016; Mohamed et al., 2022). The researchers employed a liquid crystal framework and designated these substances as Mobil

Composition of Matter or Mobil Crystalline Materials (MCM) (Grun et al., 1997; Lin & Mou, 1996). MSNs are typically classified into several categories, including Santa Barbara (SBA-1, 2, 3, 6, 12, 15, & 16), MCM (MCM-41, 48, and 50), Michigan State University (MSU), Fudan University (FDU), and Hexagonal Mesoporous Silica (HMS) (Alothman, 2012; Oo & Chatterjee, 2019; Vivero-Escoto et al., 2010). These classifications arise from the use of specific surfactants under optimal reaction conditions, which determine pore diameters (Beck et al., 1992; Danks et al., 2016). Physically, MSNs appear as fine white powder, and their porous structure can only be observed under an electron microscope (Figure 1).

Fig. 1: Circular-shaped MSN: (a) Physical appearance and (b) Enlarged 3D structure. Reproduced from Lundquist et al. (2014).

Various reaction conditions have led to the development of different members within the MCM family. MCM-41, distinguished by its use of cationic surfactants as templating agents, has become a widely utilized carrier. It features pores ranging from 2.5 to 6 nm in diameter and a 2-dimensional hexagonal shape(Munoz et al., 2003; Y. Wang et al., 2014). Another significant member, MCM-48, has a 3-dimensional cubic structure and is employed in drug carrier formulations due to its bi-continuous channels that facilitate faster ingredient transfer compared to MCM-41(Grun et al., 1997; Wang et al., 2014). MCM-50, on the other hand, adopts a lamellar configuration (Oye et al., 2001).

The symmetry of silica layered materials is influenced by the initial templating agent used during synthesis. For instance, SBA is a very structured mesoporous framework synthesised at the University of California. It features thick silica layers and larger apertures ranging from 4.6 to 30 nm) (Jarmolińska et al., 2020; Zhao et al., 1998). One templating agent, neutral copolymer alkyl poly (ethylene oxide), induces the production of cube-shaped mesopores known as SBA-11. In contrast, oligomeric surfactants result in the formation of 3-dimensional hexagon-shaped mesoporous structures called SBA-12 (Zhao et al., 1998). Moreover, this process may produce a conventional hexagon-shaped mesostructured transporter called SBA-15 and a cube-shaped cage-type configuration called SBA-16 (Feliczak-Guzik et al., 2016; Wang et al., 2009).

MSNs, including MCM-41, 48, SBA-15, and SBA-16, are extensively used in pharmaceuticals and genetics. Building on these discoveries, two trends have been identified for the four primary types of MSN, which are MCM, SBA, TUD, and KIT. Firstly, the geometrical shape of these MSN is affected by the initial templating agent employed during synthesis. Previous research suggests that a spherical shape can aid in achieving uniform dose distribution, a regulatory requirement that is challenging to meet (Mohamed et al. 2022). Therefore, it is imperative to compile a catalogue of surface templating agents, typically surfactant-based, capable of generating spherical MSNs. This can simplify regulatory compliance while optimizing the drug release profile, as the spherical shape facilitates the study of factors influencing release, drug loading percentage, and interactions with biological receptors.

Secondly, all currently produced MSNs are in powder form, indicating the need for formulators to understand the desirable characteristics of powders. This knowledge is essential for synthesizing fine, free-flowing powders suitable for industrial and bulk powder handling using high-speed machinery.

Advancement in Msn Production Technique

Considering the easily tailorable nature of mesoporous frameworks, there has been significant interest in altering the overall morphology of MSNs

to enhance their properties for diverse applications (Meng et al. 2011). Among various morphological attributes, particle diameter and shape modification play crucial roles in influencing the behaviour of the delivery system. These factors, along with surface chemistry, affect blood circulation, immune responses and delivery efficiency through specific cellular uptake pathways (Lin et al. 2010). These critical aspects and the potential for further advancements have made MSNs one of the predominant inorganic constructs for versatile delivery systems and catalysis supports.

MSN can be created using various processes, including hard or soft templates, quick self-assembling, the Stober approach, customised aerogel techniques, the hydrothermal approach, and dissolving restoration strategies. Stober pioneered a way to synthesise circular micro-sized silica nanoparticles through precise chemical procedures, later known as Stober synthesis (Stober et al., 1968). Most MSNs are made using Stober's sol-gel method, which forms colloidal fragments through hydrolysis and condensation under basic or acidic pH conditions. These colloids condense to form a three-dimensional gel state linked by cross-linked siloxane bonds (Medina et al., 2012). The common synthesis steps of MSNs can be simplified and illustrated, as shown in Figure 2.

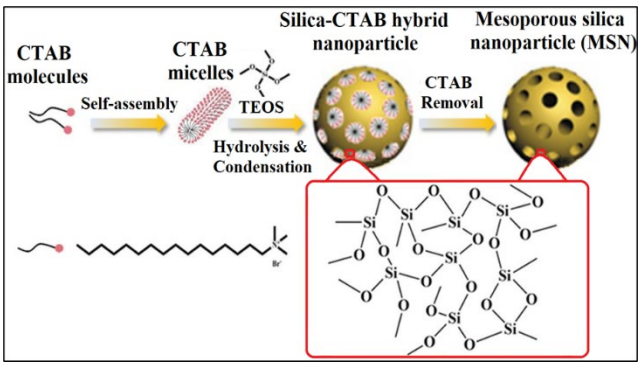

Fig. 2: Schematic illustration of the mesoporous silica synthesis from cetyltrimethylammonium bromide (CTAB) template using tetraethyl orthosilicate (TEOS) as silica precursor. Reproduced from He et al. (2020).

Apart from Stober method, diverse alterations may be applied to produce nanoparticles via distinct geometries that are exceptionally ordered and called as modified Stober method (Wang et al., 2016). This process can be improved by using a cationic surfactant precursor to generate a circular MSN framework instead of a hexagonal version yet retaining equivalent characteristics with robust and monodisperse MSN (Grun et al., 1997). The technique has a homogeneous design and controllable characteristics, making it simpler and cheaper than others. It also uses fewer excipients and is quicker (Bharti et al., 2015).

An alternative method for synthesising MSNs involves the use of hard and soft templating techniques. Biological-based templating agents are used in the soft template strategy to create porosity in MSNs, followed by heating to remove the pure mesoporous transporters (Wu et al., 2013). In hard-templating or nano-casting, capillary pressures fill templating mesopores with silica precursors. After the templating agent is removed chemically or thermally, a reverse assembly of the mesoporous silica structure is formed (Egger et al., 2015).

The main advantages for sol-gel method or Stober method are increased purity and ease of synthesis under moderate reaction conditions. In contrast, the hydrothermal technique is beneficial for obtaining MSNs with greater hydrothermal stability (Bharti et al., 2015; Shahbazi et al., 2012).

A recent modification to the synthesis of MSNs involves encapsulating drugs in hollow mesoporous silica nanoparticles (HMSNs). The large hollow cavity inside each MSN has garnered tremendous attention due to its ability to hold a high amount of drug compared to non-hollow counterparts. This unique property makes HMSNs particularly useful in cancer therapy and imaging (Chen et al. 2014).

The microwave-assisted technique is a low-cost approach for synthesizing MSNs. Various reports indicate that the method can rapidly produce MSNs with ordered pore size and arrangement (Bian et al. 2013). Another rapid, cost-effective method for the fabrication of MSNs is sonochemical synthesis. The use of photoacoustic cavitations in this process generates ordered MSNs, allowing for fine-tuning in a shorter time (Snoussi et al. 2018).

A key finding from Kankala and his team (2020) highlights advancement in three main areas:

a) Surface modification: Enhancing the MSN surface through surface engineering using various components such as polymers, liposomes, biomembranes, proteins, and metal shielding through direct immobilization or functionalization of the mesostructured surfaces.

b) Framework alteration: Modifying siliceous frameworks with various organic moieties (periodic mesoporous organosilicas) and metal species, resulting in metal-encapsulated MSNs and heterostructures.

c) Porosity changes: Adjusting the porosity of MSNs, including the development of cage-like and hollow structures. This includes hollow, yolk–shell, and core–shell architectures, resulting in different mesophases with enriched biomedical applications.

British Geologist Roderick Murchison invented a hydrothermal process for MSN synthesis to produce minerals from hot water solutions of cooling magmas. This reaction occurs in a sealed container under high pressure and temperature (Feng & Guanghua, 2011). This approach is similar to the sol-gel procedure, but the mixture is shifted into a Teflon-lined autoclave at a specific temperature, followed by template removal. The hydrothermal approach produces MSNs with higher uniformity and consistency (Jarmolińska et al., 2020; Oo et al., 2022). However, it remains uncertain at this stage whether acquiring magmas at pharmaceutical-grade quality would be feasible for biomedical or pharmaceutical applications, given the regulatory requirements for raw material quality control.

Factors Affecting Cellular Uptake and Drug Loading in MSN

Various factors affect the cellular absorption of MSNs, including particle size, pore size, shape, charge, and surface modifications. To create MSNs suitable for drug delivery, the particles must have a consistent shape and a larger pore volume, allowing for a higher drug-loading capacity. The key characteristics of MSNs are influenced by these criteria.

Particle Size

Particle size is a critical quality attribute for any product, including nanoparticles, regardless of the dosage form. It plays a significant role in delivering the encapsulated molecule to the desired target site. Smaller nanoparticles are generally more desirable for efficient drug administration due to their superior cellular absorption properties. Numerous studies on MSNs have extensively discussed factors influencing particle size, including functional organo-silanes, pH, temperature, and synthesis timing. For clinical applications of MSNs, particularly for chronic use, ultrasmall particulates (those smaller than 400 nm) are crucial to minimise toxicity. These particles must be easily transported through the circulatory system and excreted properly to avoid potential organ toxicity due to accumulation over time (Yang et al., 2018). In oncological applications, the enhanced permeability and retention (EPR) effect is a noteworthy biophysical characteristic of tumours

(Bertrand et al., 2014), where the diminutive size of nanoparticles facilitates their absorption and retention within tumour cells (Maeda, 2015). Therefore, controlling the MSN dimensions is essential to maximise therapeutic effectiveness (improving drug delivery to tumour cells) and minimise adverse reactions (favouring intravascular transport and breakdown). Researchers must ensure that particle size is well-defined for dried, reconstituted and post-reconstitution stability with a polydispersity index of less than 0.3 as targeted specification before administration.

Pore Size

The abundant mesopore channels within the MSN structure provide exceptionally spacious interior cavities, facilitating the permeation and transport of large particles such as medicines, protein molecules and nucleic acids. The types, loading quantities, and release dynamics of medicinal payloads depend on the MSN pore diameter. Smaller MSNs can only carry small drugs due to steric interference, while larger ones can transport organic substances, nanomaterials, and larger drugs. Vallet-Regí et al. observed that the bovine serum albumin uploading efficacy increased from 15% to 27% as the SBA-15 pore diameter expanded from 8.2 to 11.4 nm (Vallet-Regí et al., 2008). Reducing steric barriers by increasing pore diameter can enhance the delivery of medicinal payload through mesopores. Horcajada et al. discovered that reducing MCM-41's pore diameter from 3.6 to 2.5 nm lowered ibuprofen release in simulated body fluid (Horcajada et al., 2004). In summary, it is imperative to align the molecular weight of the therapeutic molecules with the targeted pore size of the MSNs cargo and adjust the processing parameters accordingly to achieve the desired outcome.

Hollow Structure

By adjusting the synthesis variables, MSNs may transform into homogenous or porous spheres, tubular structures, fibres, gyroids and complex networks Sun et al., 2017). Circular nanomaterials, especially hollowed ones, are being extensively developed in medical theranostics (Chen et al., 2014; Li & Shi, 2014).

Hollow structures have minimal density, huge voids, and large surfaces, making them ideal drug carriers. Incorporating pliable liquid components like an emulsion particle, vesicles, or bubbles of gas within a water-based fluid substrate creates multimodal soft-templating cores for hollow nanostructures (Li & Shi, 2014). Extraction or calcination removes the disparate central framework surfactant, forms a pattern, and produces the hollow composite. A self-generated soft-templating approach uses precursor molecule droplets as the core template, consuming the inner molecules to form outer mesostructured shells without a core template removing step.

Solid nanomaterials such as polymer latex, silica and carbon rings form cavities in the hard-templating method (Li & Shi, 2014). Depending on the solid base templates used, this method might be disparate or uniform. The approach of "structural difference-based selective etching" was first developed for constructing HMSNs by utilising the variation in structure among the silica core and the mesoporous silica layer to generate vacuous regions (Chen et al., 2010).

Chemical Composition

Mechanically stable biosystems are enabled by the inorganic Si-O-Si structure of MSNs, which facilitates effective drug delivery (Stober et al., 1968). Nevertheless, the utilisation of its chemical structure in vivo can give rise to specific limitations, such as restricted degradation as well as a singular utility (Bindini et al., 2021). Such methods for regulating structure are usually categorised by two primary approaches. The initial method entails the integration of organic and inorganic elements within the Si-O-Si structure of MSN, with the goal of achieving degradation in response to stimuli and better performance. By adding organic R subunits within a silica structure (Si-R-Si), MSN's robustness is combined with liposome and micelles' suitability. This integration results in broader application potential (Chen & Shi, 2016). The second methodology entails constructing core/shell structures to regulate the composition and structure of MSNs (Ishii et al., 2015). Hard-templating techniques promote mesoporous silica shells to

appear around bioactive inorganic nanomaterial bases (Xie et al., 2016).

Exterior/Surface Transformation

The surface properties of MSNs significantly impact their physiological behaviour. Nanomaterials with a neutral charge have a longer circulatory lifespan, whereas positively charged particles may trigger stronger immunological reactions (Nel et al., 2009). Within the last 20 years, numerous investigations into surface modification methods have greatly improved the use of MSNs in biomedicine.

To improve the in vivo biocompatibility of MSNs, they are often mixed with biodegradable polymers like polyethylene glycol (PEG). PEG reduces protein absorption and degradation due to its low interfacial energy, non-adhesive characteristics, and robust dynamics (Banche-Niclot et al., 2021; Perera et al., 2021). PEGylation is known to protect nanomaterials from accumulation, opsonisation, coagulation or in vivo scavenging, thereby extending their circulation time and enhancing their biomedical applications (Perera et al., 2021; Suk et al., 2016). Similar to other PEG-decorated nanoparticles, the scalability of these stealth MSNs is less of a concern because PEG is a well characterised Generally Recognised as Safe (GRAS) material commonly used as an excipient in large- scale pharmaceutical manufacturing.

Therapeutic Applications of MSN

Stimuli-Responsive Drug Release

Traditional cancer treatments often suffer from limited absorption and tumour resistance to chemotherapeutic agents (Park et al., 2013). MSNs are increasingly tailored with functional moieties to enable stimuli-responsive cancer therapies (He & Shi, 2014). Decorative multifunctional features around mesopore apertures can act as "sensors" (such as noble metal decorated MSNs or sugar decorated MSNs), regulating the release of therapeutic agents in response to external influences (Hosseini et al., 2023).

These mesoporous nanosystems are categorised based on the nature and triggers they respond to:

a) endogenous-triggered nanosystems respond to biological stimuli within the tumour microenvironment, such as pH, redox conditions, and proteins (Mi, 2020; Yang et al., 2018);

b) exogenous-influenced nanosystems respond to external triggers like radiation or ultrasound (Tharkar et al., 2019; B. Yang et al., 2018).

Tailored Drug Distribution

Indirect nanomaterial aggregation within tumour cells is feasible due to tumour biophysics, however, it is often insufficient for significant intratumoral deposition and effective treatment. Since cancerous tissues overstate certain biomolecules within their membrane and organelles, active targeting drug transport is widely studied (Rosenblum et al., 2018). Many agents are modified using nanotechnologies, such as MSN-based systems to accurately target malignant cells.

An additional advantage of MSNs over other inorganic nanoparticles is their relatively superior safety profile. For instance, the FDA has approved colloidal silica for use as a glidant in tablet production (Janjua et al., 2021). In addition, the widely used food additive E511 consists of amorphous silica NPs with a diameter of 100 nm. Importantly, numerous clinical trials and studies confirm the safety and efficacy of silica nanoparticles in applications such as oral drug delivery, bioimaging and photothermal therapy. Many silica-based nanoformulations have been developed, and their systematic safety evaluation is ongoing. Nonetheless, MSNs are considered more promising in the biomedical field than other inorganic nanoparticles (Bolong et al., 2023).

Gene Delivery

Substantial advancements have been achieved in enhancing the delivery of nucleic acid cargo over the past decade, leading to the creation of numerous nanocarriers with diverse structural and compositional characteristics tailored for gene transportation (Lostale-Seijo & Montenegro, 2018).

Unlike conventional chemotherapeutic drugs, gene segments are relatively large and challenging to load into typical MSNs having narrow pore sizes (< 3 nm). To overcome such constraints, two main approaches are being developed: attaching genomes to the exterior of smaller pore-sized MSNs or enclosing genes within the larger pores of MSNs by conjugation (Sun et al., 2017). Similar approaches have been previously adopted by other nanoparticles, including lipids, exosomes, polymers, polypeptides, graphene-family nanomaterials, inorganic materials, such as gold nanoparticles or their combinations (Caccamo et al. 2020). However, since genetic materials are exposed to the biological environment and liable to degradation, an excess of therapeutic molecules may be applied when designing the theoretical loading efficiency. This approach helps maintain the thermodynamic of drug release and achieve an appropriate dosage.

Additional Treatment Approaches

In addition to directly delivering the chemotherapeutic drugs or nucleic acids, MSNs are dynamically coupled with biological and chemical functionality to improve treatment efficacy. As a result, MSNs are employed in various therapeutics and diagnostics areas of biomedicine (Figure 3). Many inorganic or organic nanoparticles exhibit strong photothermal transformation, making them suitable for photothermal therapy (Wang et al., 2020). Various catalytic nanoparticles react with intratumoral H_2O_2, promoting •OH generation for chemodynamic therapy (Yang et al., 2018). Mechanically superior 3-dimensional hydrogels may facilitate osteogenesis for bone tissue engineering. Other applications include phototherapy, ultrasound therapy, radiotherapy, chemodynamic therapy, immunotherapy, tissue engineering, animal cell culture, and scaffold-based nanomaterials.

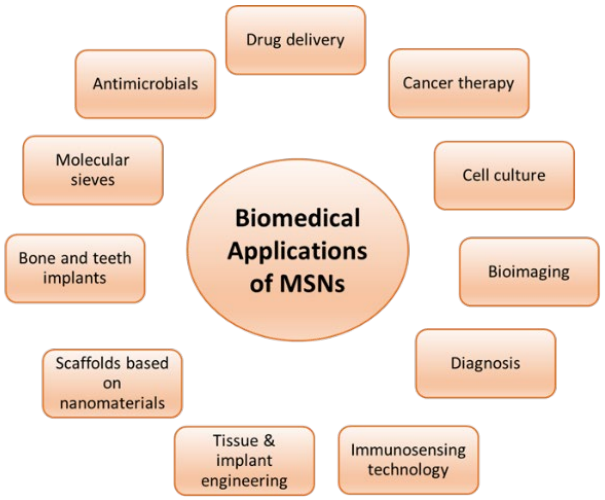

Fig. 3: Biomedical applications of mesoporous nanoparticles. Reproduced from Ahmed et al. (2022), Mi (2020), and Tharkar et al. (2019).

Biomedical Applications of MSN

Delivery of Bioactive Molecules

MSNs are considered to have great potential as nanocarriers for drug delivery due to their unique characteristics (Vivero-Escoto et al., 2010; Wang & Kohane, 2017). Their features encompass a permeable framework for better utilisation of material, resistance to chemical changes, surface properties that promote equilibrium, compliance with biological systems, and a capacity to dissipate substances in a regulated manner regardless of external triggers, including the ability to mark certain cells specifically (Hosseini et al., 2023; Mi, 2020). MSNs showed the potential for encapsulating and controlling the flow of diverse bioactive compounds, including chemotherapy agents, genetic material, growth stimulants and catalysts (Ahmed et al., 2022). MSNs can inhibit biological substances from diffusing prematurely into the channels by functionalising multiple receptors.

Regulate Chemotherapy Release of Medication

MSN-based carriers are being utilised for dispensing chemotherapy medications like DOX, camptothecin, 5-F- Erlotinib, methotrexate, irinotecan, cisplatin and banoxantrone along with RNA particles (Liu et al., 2018; Wu et al., 2018). Despite MSNs having great medication transporting ability because of product dispersion, MSNs could fail to maintain or regulate cytotoxic medication distribution and thus may cause general impairment of natural tissues. Thus, different methods have emerged to mitigate encapsulated medication burst discharge. MSN can be functionalised with agents that respond to stimuli such as light, temperature, electromagnetic fields, pH, catalysts, reactive oxygen species or specific ligands. These functionalised MSNs can selectively release cytotoxic drugs and target specific types of tissues.

Disease Treatment

MSN-based nanocarriers have demonstrated promise in addressing conditions such as Alzheimer's disease and heart failure. The onset of Alzheimer's disease, linked to metallic ions that accelerate Aβ accumulation and ROS development, can be mitigated by metal chelators (Leyane et al., 2022). For instance, phenylboronic acid-functionalised MSNs carrying β-D-glucose-AuNPs with clioquinol (CQ) showed H2O2-regulated distribution within the dementia surroundings. By inhibiting Aβ accumulation, CQ reduced phagocytosis within dementia-related PC12 cells (Yang et al., 2016). In zebrafish studies on acute killer red (SqKR15)-based ROS-triggered heart failure. MSN-based nanoparticles delivered curcumin or captopril locally, improving pulse and heart rate. Additionally, glucose-responsive customised MSNs have shown potential in regulating blood sugar levels in diabetic animal models (Hou et al., 2018).

Lesion Restoration and Tissue Regrowth

MSN-based carriers have shown promise in healing lesions and promoting tissue regrowth. Increased ROS levels in damaged regions can trigger cell death, fibrosis and inflammation. ROS-responsive MSN-based carriers have facilitated improved wound repair (Wang et al., 2016). For instance, a flexible ROS-scavenging composite was created by doping amino-functionalised MSN with ultra-small ceria nanocrystals. This composite enhanced epidermal outgrowth and decreased scarring by strengthening tissue adhesion and accelerating the recovery process. In addition, hepatocyte nuclear factor 3β plasmid DNA (pHNF3β) delivered via a positive-charged MSN-based nanosystem significantly promoted the differentiation of induced pluripotent stem cells into functional hepatocyte-like cells within just two weeks of in vitro study. Enhancing the delivery rate of pHNF3β further improved the cell differentiating process (Wu et al., 2018).

Medical Imaging and Diagnostic Applications

To minimise overall cytotoxicity, localised and customised anticancer administration strategies are essential. Using nanocarriers such as FITC, Hoechst, carbon dots, 64Cu, fluorescein, multicolour up-conversion nanoparticles, chlorin e6, and N,N-phenylenebis (salicylideneimine) dicarboxylic acid (Salphdc) facilitates targeted delivery and distribution monitoring of cancer treatments (Lai et al., 2015; Mi, 2020). In particular, nanoparticles incorporating DOX-loaded 64Cu-labeled MSNs, controlled by AuNPs, provide near-infrared (NIR) triggered DOX release and a combined photothermal therapeutic response against cancer. Additionally, these nanocarriers also serve as bio-tools for PET imaging, capable of detecting clinically significant lung tumours spontaneously in mice with urethane-induced lung cancer (Cheng et al., 2016).

Biocatalysis

MSNs are excellent biocatalysts due to their large surface area, durability, uniform porosity, and good functionalisation. They shield catalysts against proteolysis and reduce immune response, enabling intracellular bioanalysis. For instance, a self-catalysed luminescence nanosystem has been developed with luciferin placed inside MSN pores, AuNPs providing capping components through disulfide bonds and PEGlyated luciferase, designed for assessing tumour progression (Sun et al., 2011).

Another approach involves the in-situ creation of AuNPs on amino-functionalised MSNs to produce an efficient nanoreactor exhibiting enzyme-mimetic catalytic characteristics, allowing a chain reaction via a self-activated process. This method can develop

synthetic catalysts with varied functions and reactivities for biocatalysis, bioassays, nano-biomedicine, and nanotechnology (Lin et al., 2013).

Other Applications

Biosensors, bioassays, and antimicrobial activities utilise modified MSNs to be nanocarriers which show extremely precise affinity for attached catalysts via His-tag_Ni ion coupling. Identifying histidine-tagged catalysts is easy and has a high throughput with such substances, which can separate and immobilise a variety of polyhistidine-tagged proteins (Raducanu et al., 2020). MSNs modified using phenyltrimethyl moieties may trap thymophthalein, a pH marker for preferential monitoring of prostate-specific agents, offering a cheap, fast way of screening biomarkers within intricate specimens. AuNPs-modified MSNs containing antibiotic-loaded nano-vehicles had combinatorial activities, suggesting drug-resistant illness therapy (Wang et al., 2016).

Safety, Biodistribution and Fate of MSN

MSNs represent exciting biological vehicles that prevent premature release of medicines and enhance their stability (Hosseinpour et al., 2021). Current research focuses on the surface remodelling of MSNs by customising functional domains externally or internally to improve drug uptake and release at targeted regions (Li & Shi, 2014; Mohamed et al., 2022). MSNs can be paired with polymeric molecules, chemical compounds, or nucleic acids to produce hybrids with various biological functions, primarily for tailored medication delivery and controlled release profiles (Lostale-Seijo & Montenegro, 2018; Sun et al., 2017).

Although MSNs have several biomedical applications, the US FDA has yet to authorise their use until their fate, biodistribution, and clearances are well understood (Lérida-Viso et al., 2023). MSNs tend to accumulate in the reticuloendothelial system, particularly in the organs associated with liver function, because of protein adsorption on their surface, usually from serum(Zhang et al., 2021). These issues can be resolved by coating the MSN surface with a hydrophilic polymer like PEG (Banche-Niclot et al., 2021; Perera et al., 2021; Suk et al., 2016). Protein absorption affects hepatobiliary evacuation and bile discharge, whereas finer particles tend to aggregate or accumulate in faeces. Studies have also demonstrated enhanced renal clearance of these nanoparticles.

Conclusion

In modern medicine, MSNs have revolutionised both diagnosis and therapy by enhancing drug stability and addressing issues related to low drug solubility. However, their clinical application is complicated by immunogenicity and toxicity due to the accumulation of inorganic substances. A major barrier to the use of MSNs for biomedical delivery is the lack of understanding of their long-term safety. In addition, the inconsistency in characterisation procedures and toxicity assessment tools hinders their commercialisation and clinical use. Nevertheless, with growing knowledge of MSNs' behaviour in the body and advancement in their formulation, MSNs are expected to reach therapeutic applications soon.

Authors contributions

The authors contributed to this review article in the following ways:

Fatema Tuz Zohera (First author): Articles collection, content analysis, and main content writer of the manuscript.

MD. Abul Kalam Azad: Manuscript editing, providing valuable insights, and interpretation of relevant articles for the manuscript.

May Kyaw Oo: Manuscript editing, referencing, and formatting for submission.

Farahidah Mohamed (Corresponding author): Conceptualisation and critical revision of the manuscript for intellectual content, providing valuable insights, and ensuring accuracy in the presentation of information.

All authors have reviewed and approved the final version of the manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

References

Ahmed, H., Gomte, S. S., Prathyusha, E., A, P., Agrawal, M., & Alexander, A. (2022). Biomedical applications of mesoporous silica nanoparticles as a drug delivery carrier. *Journal of Drug Delivery Science and Technology*, *76*, 103729. https://doi.org/10.1016/j.jddst.2022.103729

Alothman, Z. A. (2012). Fundamental aspects of silicate mesoporous materials. *Materials*, *5*(12), 2874–2902.

Alyassin, Y., Sayed, E. G., Mehta, P., Ruparelia, K., Arshad, M. S., Rasekh, M., Shepherd, J., Kucuk, I., Wilson, P. B., Singh, N., Chang, M.-W., Fatouros, D. G., & Ahmad, Z. (2020). Application of mesoporous silica nanoparticles as drug delivery carriers for chemotherapeutic agents. *Drug Discovery Today*, *25*(8), 1513–1520. https://doi.org/10.1016/j.drudis.2020.06.006

Atanase, L. I. (2021). Micellar drug delivery systems based on natural biopolymers. *Polymers*, *13*(3), 477. https://doi.org/10.3390/polym13030477

Banche-Niclot, F., Montalbano, G., Fiorilli, S., & Vitale-Brovarone, C. (2021). PEG-coated large mesoporous silicas as smart platform for protein delivery and their use in a collagen-based formulation for 3D printing. *International Journal of Molecular Sciences*, *22*(4), 1718. https://doi.org/10.3390/ijms22041718

Beck, J. S., Vartuli, J. C., Roth, W. J., Leonowicz, M. E., Kresge, C. T., Schmitt, K. D., Chu, C. T. W., Olson, D. H., Sheppard, E. W., McCullen, S. B., Higgins, J. B., & Schlenker, J. L. (1992). A new family of mesoporous molecular sieves prepared with liquid crystal templates. *Journal of the American Chemical Society*, *114*(27), 10834–10843. https://doi.org/10.1021/ja00053a020

Begines, B., Ortiz, T., Perez-Aranda, M., Martínez, G., Merinero, M., Arguelles-Arias, F., & Alcudia, A. (2020). Polymeric nanoparticles for drug delivery: Recent developments and future prospects. *Nanomaterials*, *10*(7), 1403. https://doi.org/10.3390/nano10071403

Bertrand, N., Wu, J., Xu, X., Kamaly, N., & Farokhzad, O. C. (2014). Cancer nanotechnology: The impact of passive and active targeting in the era of modern cancer biology. *Advanced Drug Delivery Reviews*, *66*, 2–25. https://doi.org/10.1016/j.addr.2013.11.009

Bharti, C., Gulati, N., Nagaich, U., & Pal, A. (2015). Mesoporous silica nanoparticles in target drug delivery system: A review. *International Journal of Pharmaceutical Investigation*, *5*(3), 124. https://doi.org/10.4103/2230-973X.160844

Bian, S., Gao, K., Shen, H., Jiang, X., Long, Y., & Chen, Y. (2013). Organic/inorganic hybrid mesoporous silica membrane rapidly synthesized by a microwave-assisted method and its application in enzyme adsorption and electrocatalysis. *Journal of Materials Chemistry B*, *1*, 3267-3276. https://doi.org/10.1039/C3TB20169D

Bindini, E., Ramirez, M. de los A., Rios, X., Cossío, U., Simó, C., Gomez-Vallejo, V., Soler-Illia, G., Llop, J., & Moya, S. E. (2021). In vivo tracking of the degradation of mesoporous silica through 89 Zr radio-labeled core–shell nanoparticles. *Small*, *17*(30). https://doi.org/10.1002/smll.202101519

Xu, B., Li, S., Shi, R., Liu, H. (2023). Multifunctional mesoporous silica nanoparticles for biomedical applications. *Signal Transduction and Targeted Therapy, 8*, Article 435. https://doi.org/10.1038/s41392-023-01654-7

Caccamo, D., Currò, M., Ientile, R., et al. (2020). Intracellular fate and impact on gene expression of doxorubicin/cyclodextrin-graphene nanomaterials at sub-toxic

concentration. *International Journal of Molecular Sciences*, *21*, 4891. https://doi.org/10.3390/ijms21144891

Chen, Y., Chen, H., Guo, L., He, Q., Chen, F., Zhou, J., Feng, J., & Shi, J. (2010). Hollow/rattle-type mesoporous nanostructures by a structural difference-based selective etching strategy. *ACS Nano*, *4*(1), 529–539. https://doi.org/10.1021/nn901398j

Chen, Y., Meng, Q., Wu, M., Wang, S., Xu, P., Chen, H., Li, Y., Zhang, L., Wang, L., & Shi, J. (2014). Hollow mesoporous organosilica nanoparticles: A generic intelligent framework-hybridization approach for biomedicine. *Journal of the American Chemical Society*, *136*(46), 16326–16334. https://doi.org/10.1021/ja508721y

Chen, Y., & Shi, J. (2016). Chemistry of mesoporous organosilica in nanotechnology: Molecularly organic–inorganic hybridization into frameworks. *Advanced Materials*, *28*(17), 3235–3272. https://doi.org/10.1002/adma.201505147

Chen, F., Hong, H., Shi, S., Goel, S., Valdovinos, H. F., Hernandez, R., Theuer, C. P., Barnhart, T. E., & Cai, W. (2015). Engineering of hollow mesoporous silica nanoparticles for remarkably enhanced tumor active targeting efficacy. *Scientific Reports*, *4*, 5080. https://doi.org/10.1038/srep05080

Cheng, B., He, H., Huang, T., Berr, S. S., He, J., Fan, D., Zhang, J., & Xu, P. (2016). Gold nanosphere gated mesoporous silica nanoparticle responsive to near-infrared light and redox potential as a theranostic platform for cancer therapy. *Journal of Biomedical Nanotechnology*, *12*(3), 435–449. https://doi.org/10.1166/jbn.2016.2195

Danks, A. E., Hall, S. R., & Schnepp, Z. (2016). The evolution of 'sol–gel' chemistry as a technique for materials synthesis. *Materials Horizons*, *3*(2), 91–112. https://doi.org/10.1039/C5MH00260E

Egger, S. M., Hurley, K. R., Datt, A., Swindlehurst, G., & Haynes, C. L. (2015). Ultraporous mesostructured silica nanoparticles. *Chemistry of Materials*, *27*(9), 3193–3196. https://doi.org/10.1021/cm504448u

Fadeel, B., & Garcia-Bennett, A. E. (2010). Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. *Advanced Drug Delivery Reviews*, *62*(3), 362–374. https://doi.org/10.1016/j.addr.2009.11.008

Feliczak-Guzik, A., Jadach, B., Piotrowska, H., Murias, M., Lulek, J., & Nowak, I. (2016). Synthesis and characterization of SBA-16 type mesoporous materials containing amine groups. *Microporous and Mesoporous Materials*, *220*, 231–238.

Feng, S., & Guanghua, L. (2011). Hydrothermal and solvothermal syntheses. In *Modern Inorganic Synthetic Chemistry* (pp. 63–95). Elsevier. https://doi.org/10.1016/B978-0-444-53599-3.10004-6

Grun, M., Lauer, I., & Unger, K. K. (1997). The synthesis of micrometer- and submicrometer-size spheres of ordered mesoporous oxide MCM-41. *Advanced Materials*, *9*(3), 254–257. https://doi.org/10.1002/adma.19970090317

He, Q., & Shi, J. (2014). MSN anti-cancer nanomedicines: Chemotherapy enhancement, overcoming of drug resistance, and metastasis inhibition. *Advanced Materials*, *26*(3), 391–411. https://doi.org/10.1002/adma.201303123

He, Y., Shu, C.-C., Guo, Y., Long, M., & Xu, H. (2020). Visualizing ultrasmall silica–CTAB hybrid nanoparticles for generating high photoluminescence. *Journal of Materials Chemistry C*, *8*(19), 6413–6421. https://doi.org/10.1039/D0TC00797H

Horcajada, P., Rámila, A., Pérez-Pariente, J., & Vallet-Regí, M. (2004). Influence of pore size of MCM-41 matrices on drug delivery rate. *Microporous and Mesoporous Materials*, *68*(1–

3), 105–109. https://doi.org/10.1016/j.micromeso.2003.12.012

Hosseini, S. M., Mohammadnejad, J., Salamat, S., Beiram Zadeh, Z., Tanhaei, M., & Ramakrishna, S. (2023). Theranostic polymeric nanoparticles as a new approach in cancer therapy and diagnosis: a review. *Materials Today Chemistry*, *29*, 101400. https://doi.org/10.1016/j.mtchem.2023.101400

Hosseinpour, S., Cao, Y., Liu, J., Xu, C., & Walsh, L. J. (2021). Efficient transfection and long-term stability of rno-miRNA-26a-5p for osteogenic differentiation by large pore sized mesoporous silica nanoparticles. *Journal of Materials Chemistry B*, *9*(9), 2275–2284. https://doi.org/10.1039/D0TB02756A

Hou, L., Zheng, Y., Wang, Y., Hu, Y., Shi, J., Liu, Q., Zhang, H., & Zhang, Z. (2018). Self-regulated carboxyphenylboronic acid-modified mesoporous silica nanoparticles with "Touch Switch" releasing property for insulin delivery. *ACS Applied Materials & Interfaces*, *10*(26), 21927–21938. https://doi.org/10.1021/acsami.8b06998

Meng, H., Xue, M., Xia, T., Ji, Z., Tarn, D. Y., Zink, J. I., & Nel, A. E. (2011). Use of size and a copolymer design feature to improve the biodistribution and the enhanced permeability and retention effect of doxorubicin-loaded mesoporous silica nanoparticles in a murine xenograft tumor model. *ACS Nano, 5*(5), 4131. https://doi.org/10.1021/nn200809t

Ishii, H., Ikuno, T., Shimojima, A., & Okubo, T. (2015). Preparation of core–shell mesoporous silica nanoparticles with bimodal pore structures by regrowth method. *Journal of Colloid and Interface Science*, *448*, 57–64. https://doi.org/10.1016/j.jcis.2015.01.057

Janjua, T. I., Cao, Y., Yu, C., et al. (2021). Clinical translation of silica nanoparticles. *Nature Reviews Materials, 6*, 1072-1074. https://doi.org/10.1038/s41578-021-00385-x

Jarmolińska, S., Feliczak-Guzik, A., & Nowak, I. (2020). Synthesis, characterization and use of mesoporous silicas of the following types SBA-1, SBA-2, HMM-1 and HMM-2. *Materials*, *13*(19), 1–33.

Lai, J., Shah, B. P., Zhang, Y., Yang, L., & Lee, K.-B. (2015). Real-time monitoring of ATP-responsive drug release using mesoporous-silica-coated multicolor upconversion nanoparticles. *ACS Nano*, *9*(5), 5234–5245. https://doi.org/10.1021/acsnano.5b00641

Lérida-Viso, A., Estepa-Fernández, A., García-Fernández, A., Martí-Centelles, V., & Martínez-Máñez, R. (2023). Biosafety of mesoporous silica nanoparticles; towards clinical translation. *Advanced Drug Delivery Reviews*, *201*, 115049. https://doi.org/10.1016/j.addr.2023.115049

Leyane, T. S., Jere, S. W., & Houreld, N. N. (2022). Oxidative stress in ageing and chronic degenerative pathologies: Molecular mechanisms involved in counteracting oxidative stress and chronic inflammation. *International Journal of Molecular Sciences*, *23*(13), 7273. https://doi.org/10.3390/ijms23137273

Li, Y., & Shi, J. (2014). Hollow-structured mesoporous materials: Chemical synthesis, functionalization and applications. *Advanced Materials*, *26*(20), 3176–3205. https://doi.org/10.1002/adma.201305319

Lin, H. P., & Mou, C. Y. (1996). Tubules-within-a-tubule hierarchical order of mesoporous molecular sieves in MCM-41. *Science*, *273*(5276), 765–768. https://doi.org/10.1126/science.273.5276.765

Lin, Y., Li, Z., Chen, Z., Ren, J., & Qu, X. (2013). Mesoporous silica-encapsulated gold nanoparticles as artificial enzymes for self-activated cascade catalysis. *Biomaterials*, *34*(11), 2600–2610. https://doi.org/10.1016/j.biomaterials.2013.01.007

Liu, J., Liu, X., Yuan, Y., Li, Q., Chang, B., Xu, L., Cai, B., Qi, C., Li, C., Jiang, X., Wang, G., Wang, Z., & Wang, L. (2018). Supramolecular modular approach toward conveniently constructing and multifunctioning a pH/Redox dual-responsive drug delivery nanoplatform for improved cancer chemotherapy. *ACS Applied Materials & Interfaces*, *10*(31), 26473–26484. https://doi.org/10.1021/acsami.8b05232

Lostale-Seijo, I., & Montenegro, J. (2018). Synthetic materials at the forefront of gene delivery. *Nature Reviews Chemistry*, *2*(10), 258–277. https://doi.org/10.1038/s41570-018-0039-1

Lundquist, C., Loo, C., Meraz, I., Cerda, J., Liu, X., & Serda, R. (2014). Characterization of free and porous silicon-encapsulated superparamagnetic iron oxide nanoparticles as platforms for the development of theranostic vaccines. *Medical Sciences*, *2*(1), 51–69. https://doi.org/10.3390/medsci2010051

Maeda, H. (2015). Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity. *Advanced Drug Delivery Reviews*, *91*, 3–6. https://doi.org/10.1016/j.addr.2015.01.002

Mandal, A. K. (2021). Dendrimers in targeted drug delivery applications: A review of diseases and cancer. *International Journal of Polymeric Materials and Polymeric Biomaterials*, *70*(4), 287–297. https://doi.org/10.1080/00914037.2020.1713780

Manzano, M., & Vallet-Regí, M. (2020). Mesoporous silica nanoparticles for drug delivery. *Advanced Functional Materials*, *30*(2). https://doi.org/10.1002/adfm.201902634

Medina, C., Medina, C., Jacoby, Malinski, Radomski, M. W., & Corbalan, J. J. (2012). Amorphous silica nanoparticles aggregate human platelets: Potential implications for vascular homeostasis. *International Journal of Nanomedicine*, 631. https://doi.org/10.2147/IJN.S28293

Mi, P. (2020). Stimuli-responsive nanocarriers for drug delivery, tumor imaging, therapy and theranostics. *Theranostics*, *10*(10), 4557–4588. https://doi.org/10.7150/thno.38069

Mohamed, F., Oo, M. K., Chatterjee, B., & Alallam, B. (2022). Biocompatible supramolecular mesoporous silica nanoparticles as the next-generation drug delivery system. *Frontiers in Pharmacology*, *13*. https://doi.org/10.3389/fphar.2022.886981

Munoz, B., Rámila, A., Pariente, J. P., Díaz, I., & Regí, M. V. (2003). MCM-41 organic modification as drug delivery rate regulator. *Chemistry of Materials*, *15*(2), 500–503. https://doi.org/10.1021/cm021217q

Nel, A. E., Madler, L., Velegol, D., Xia, T., Hoek, E. M. V., Somasundaran, P., Klaessig, F., Castranova, V., & Thompson, M. (2009). Understanding biophysicochemical interactions at the nano–bio interface. *Nature Materials*, *8*(7), 543–557. https://doi.org/10.1038/nmat2442

Oo, M. K., Alallam, B., Doolaanea, A. A., Khatib, A., Mohamed, F., & Chatterjee, B. (2022). Exploring the effect of glycerol and hydrochloric acid on mesoporous silica synthesis: Application in insulin loading. *ACS Omega*, *7*(31), 27126–27134. https://doi.org/10.1021/acsomega.2c01386

Oo, M. K., & Chatterjee, B. (2019). Issues and challenges of orally-administered mesoporous silica-based drug delivery systems. *Journal of Pharmaceutical Sciences and Technology Management*, *3*(1), 12–22.

Oye, G., Sjoblom, J., & Stocker, M. (2001). Synthesis, characterization and potential applications of new materials in the mesoporous range. *Advances in Colloid and Interface Science*, *89–90*, 439–466. https://doi.org/10.1016/S0001-8686(00)00066-X

Park, S. B., Goldstein, D., Krishnan, A. V., Lin, C. S., Friedlander, M. L., Cassidy, J., Koltzenburg, M., & Kiernan, M. C. (2013). Chemotherapy-induced peripheral neurotoxicity: A critical analysis. *CA: A Cancer Journal for Clinicians*, *63*(6), 419–437. https://doi.org/10.3322/caac.21204

Perera, Y. R., Xu, J. X., Amarasekara, D. L., Hughes, A. C., Abbood, I., & Fitzkee, N. C. (2021). Understanding the adsorption of peptides and proteins onto PEGylated gold nanoparticles. *Molecules*, *26*(19), 5788. https://doi.org/10.3390/molecules26195788

Plaza-Oliver, M., Santander-Ortega, M. J., & Lozano, M. Victoria. (2021). Current approaches in lipid-based nanocarriers for oral drug delivery. *Drug Delivery and Translational Research*, *11*(2), 471–497. https://doi.org/10.1007/s13346-021-00908-7

Raducanu, V.-S., Isaioglou, I., Raducanu, D.-V., Merzaban, J. S., & Hamdan, S. M. (2020). Simplified detection of polyhistidine-tagged proteins in gels and membranes using a UV-excitable dye and a multiple chelator head pair. *Journal of Biological Chemistry*, *295*(34), 12214–12223. https://doi.org/10.1074/jbc.RA120.014132

Kankala, R. K., Han, Y.-H., Na, J., Lee, C.-H., Sun, Z., Wang, S.-B., Kimura, T., Ok, Y. S., Yamauchi, Y., Chen, A.-Z., & Wu, K. C.-W. (2020). Nanoarchitectured structure and surface biofunctionality of mesoporous silica nanoparticles. *Advanced Materials, 1907035*. https://doi.org/10.1002/adma.201907035

Rosenblum, D., Joshi, N., Tao, W., Karp, J. M., & Peer, D. (2018). Progress and challenges towards targeted delivery of cancer therapeutics. Nature Communications, 9(1), 1410. https://doi.org/10.1038/s41467-018-03705-y

Scicluna, M. C., & Vella-Zarb, L. (2020). Evolution of nanocarrier drug-delivery systems and recent advancements in covalent organic framework-drug systems. ACS Applied Nano Materials, 3(4), 3097–3115. https://doi.org/10.1021/acsanm.9b02603

Snoussi, Y., Bastide, S., Abderrabba, M., & Chehimi, M. M. (2018). Sonochemical synthesis of Fe3O4@NH2-mesoporous silica@Polypyrrole/Pd: A core/double shell nanocomposite for catalytic applications. *Ultrasonics Sonochemistry, 41*, 551-561. https://doi.org/10.1016/j.ultsonch.2017.10.021

Stober, W., Fink, A., & Bohn, E. (1968). Controlled growth of monodisperse silica spheres in the micron size range. Journal of Colloid and Interface Science, 26(1), 62–69. https://doi.org/10.1016/0021-9797(68)90272-5

Suk, J. S., Xu, Q., Kim, N., Hanes, J., & Ensign, L. M. (2016). PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Advanced Drug Delivery Reviews, 99, 28–51. https://doi.org/10.1016/j.addr.2015.09.012

Sun, L., Wang, D., Chen, Y., Wang, L., Huang, P., Li, Y., Liu, Z., Yao, H., & Shi, J. (2017). Core-shell hierarchical mesostructured silica nanoparticles for gene/chemo-synergetic stepwise therapy of multidrug-resistant cancer. Biomaterials, 133, 219–228. https://doi.org/10.1016/j.biomaterials.2017.04.028

Sun, X., Zhao, Y., Lin, V. S.-Y., Slowing, I. I., & Trewyn, B. G. (2011). Luciferase and luciferin co-immobilized mesoporous silica nanoparticle materials for intracellular biocatalysis. Journal of the American Chemical Society, 133(46), 18554–18557. https://doi.org/10.1021/ja2080168

Tawfeek, G. M., Baki, M. H. A., Ibrahim, A. N., et al. (2019). Enhancement of the therapeutic efficacy of praziquantel in murine Schistosomiasis mansoni using silica nanocarrier. *Parasitology Research, 118*(12), 3519-3533. https://doi.org/10.1007/s00436-019-06475-8

Tharkar, P., Varanasi, R., Wong, W. S. F., Jin, C. T., & Chrzanowski, W. (2019). Nano-enhanced drug delivery and therapeutic ultrasound for cancer treatment and beyond. *Frontiers in Bioengineering and Biotechnology*, *7*. https://doi.org/10.3389/fbioe.2019.00324

Vallet-Regí, M., Balas, F., Colilla, M., & Manzano, M. (2008). Bone-regenerative bioceramic implants with drug and protein-controlled delivery capability. *Progress in Solid State Chemistry*, *36*(3), 163–191. https://doi.org/10.1016/j.progsolidstchem.2007.10.002

Vivero-Escoto, J. L., Slowing, I. I., Trewyn, B. G., & Lin, V. S. Y. (2010). Mesoporous silica nanoparticles for intracellular controlled drug delivery. *Small*, *6*(18), 1952–1967. https://doi.org/10.1002/smll.200901789

Wang, J., Wu, X., Shen, P., Wang, J., Shen, Y., Shen, Y., Webster, T. J., & Deng, J. (2020). Applications of inorganic nanomaterials in photothermal therapy based on combinational cancer treatment. *International Journal of Nanomedicine*, *Volume 15*, 1903–1914. https://doi.org/10.2147/IJN.S239751

Wang, Y., Ding, X., Chen, Y., Guo, M., Zhang, Y., Guo, X., & Gu, H. (2016). Antibiotic-loaded, silver core-embedded mesoporous silica nanovehicles as a synergistic antibacterial agent for the treatment of drug-resistant infections. *Biomaterials*, *101*, 207–216. https://doi.org/10.1016/j.biomaterials.2016.06.004

Wang, Y., & Kohane, D. S. (2017). External triggering and triggered targeting strategies for drug delivery. *Nature Reviews Materials*, *2*(6), 17020. https://doi.org/10.1038/natrevmats.2017.20

Wang, Y., Sun, L., Jiang, T., Zhang, J., Zhang, C., Sun, C., Deng, Y., Sun, J., & Wang, S. (2014). The investigation of MCM-48-type and MCM-41-type mesoporous silica as oral solid dispersion carriers for water insoluble cilostazol. *Drug Development and Industrial Pharmacy*, *40*(6), 819–828.

Wang, Y., Zhang, F., Wang, Y., Ren, J., Li, C., Liu, X., Guo, Y., Guo, Y., & Lu, G. (2009). Synthesis of length controllable mesoporous SBA-15 rods. *Materials Chemistry and Physics*, *115*, 649–655.

Wu, H., Li, F., Wang, S., Lu, J., Li, J., Du, Y., Sun, X., Chen, X., Gao, J., & Ling, D. (2018). Ceria nanocrystals decorated mesoporous silica nanoparticle-based ROS-scavenging tissue adhesive for highly efficient regenerative wound healing. *Biomaterials*, *151*, 66–77. https://doi.org/10.1016/j.biomaterials.2017.10.018

Wu, M., Lin, X., Tan, X., Li, J., Wei, Z., Zhang, D., Zheng, Y., Zheng, A., Zhao, B., Zeng, Y., Liu, X., & Liu, J. (2018). Photoresponsive nanovehicle for two independent wavelength light-triggered sequential release of P-gp shRNA and doxorubicin to optimize and enhance synergistic therapy of multidrug-resistant cancer. *ACS Applied Materials & Interfaces*, *10*(23), 19416–19427. https://doi.org/10.1021/acsami.8b03823

Wu, S. H., Mou, C. Y., & Lin, H. P. (2013). Synthesis of mesoporous silica nanoparticles. *Chemical Society Reviews*, *42*(9), 3862. https://doi.org/10.1039/c3cs35405a

Xie, Y., Kocaefe, D., Chen, C., & Kocaefe, Y. (2016). Review of Research on Template Methods in Preparation of Nanomaterials. *Journal of Nanomaterials*, *2016*.

Yang, B., Chen, Y., & Shi, J. (2018). Exogenous/endogenous-triggered mesoporous silica cancer nanomedicine. *Advanced Healthcare Materials*, *7*(20). https://doi.org/10.1002/adhm.201800268

Yang, L., Yin, T., Liu, Y., Sun, J., Zhou, Y., & Liu, J. (2016). Gold nanoparticle-capped mesoporous silica-based H2O2-responsive controlled release system for Alzheimer's disease treatment. *Acta Biomaterialia*, *46*, 177–190. https://doi.org/10.1016/j.actbio.2016.09.010

Lin, Y. S., & Haynes, C. L. (2010). Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *Journal of the American Chemical Society*, *132*, 4834. https://doi.org/10.1021/ja910846q

Yu, F., Wu, H., Tang, Y., et al. (2018). Temperature-sensitive copolymer-coated fluorescent mesoporous silica nanoparticles as a reactive oxygen species activated drug delivery system. *International Journal of Pharmaceutics*, *536*(1), 11-20. https://doi.org/10.1016/j.ijpharm.2017.11.025

Zhang, M., Gao, S., Yang, D., Fang, Y., Lin, X., Jin, X., Liu, Y., Liu, X., Su, K., & Shi, K. (2021). Influencing factors and strategies of enhancing nanoparticles into tumors in vivo. *Acta Pharmaceutica Sinica B*, *11*(8), 2265–2285. https://doi.org/10.1016/j.apsb.2021.03.033

Zhang, W., Zheng, N., Chen, L., Xie, L., Cui, M., Li, S., & Xu, L. (2018). Effect of shape on mesoporous silica nanoparticles for oral delivery of indomethacin. *Pharmaceutics*, *11*(1), 4. https://doi.org/10.3390/pharmaceutics11010004

Zhao, D., Feng, J., Huo, Q., Melosh, N., Fredrickson, G. H., Chmelka, B. F., & Stucky, G. D. (1998). Triblock copolymer syntheses of mesoporous silica with periodic 50 to 300 Angstrom pores. *Science*, *279*(5350), 548–552. https://doi.org/10.1126/science.279.5350.548

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A comprehensive review of the use of plant-derived antioxidants in the management of non-alcoholic liver toxicity

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Abstract

With its rising incidence, non-alcoholic fatty liver disease (NAFLD) has become a global health problem. Hepatic lipid buildup, inflammation, and oxidative stress result from complex interactions between metabolic, genetic, and environmental variables in the development of nonalcoholic fatty liver disease. The potential significance of antioxidants generated from phytochemicals in alleviating non-alcoholic liver damage has garnered substantial interest among the many treatment methods. The goal of this thorough review is to summarize and assess the body of research on the application of antiin in the treatment of non-alcoholic fatty liver disease. A comprehensive examination of peer-reviewed research from many databases demonstrates various phytochemicals with antioxidant characteristics and their possible effects on oxidative stress, inflammation, and hepatic lipid metabolism. Prominent phytochemicals such as curcumin, resveratrol, quercetin, silymarin, and green tea catechins are among those whose antioxidative mechanisms are included in the review. Preclinical and clinical research on these substances has revealed encouraging results, suggesting that they may be able to lessen inflammation and hepatic steatosis while also enhancing liver function. We investigate the molecular mechanisms underlying their protective benefits, including reduction of pro-inflammatory cytokines and modification of nuclear factor-erythroid 2-related factor 2 (Nrf2). The study also discusses the shortcomings and difficulties in the existing research, highlighting the necessity of more clinical trials, standardized dosing schedules, and research into the possible synergistic effects of mixing several phytochemicals. To present a fair picture of the therapeutic application of phytochemical antioxidants, safety issues, and possible negative effects are also included. This review emphasizes the potential use of plant phytochemical-derived antioxidants in the treatment of Non-alcoholic steatohepatitis, and non-alcoholic hepatic damage. To provide precise guidelines and maximize their therapeutic potential in the setting of non-alcoholic liver damage or liver toxicity, more investigation and clinical data are necessary.

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Introduction

Obesity, caused by high-fat diets, is a common cause of non-alcoholic fatty liver disease (NAFLD), which is becoming a universal cause of liver disease worldwide, particularly in Western countries. Despite its high prevalence, only a small proportion of affected individuals will become inflamed, followed by fibrosis and chronic liver diseases, and most patients only show simple steatosis. Various mechanisms have been proposed for liver damage, including endoplasmic reticulum stress, perturbation of autophagy, mitochondrial dysfunction, hepatocellular apoptosis, gut microbiota imbalance, dysregulation of microRNAs, genetic/epigenetic risk factors, and an increase in inflammatory responses. These proposed mechanisms allow for a variety of hits acting together on subjects to mediate and offer a more accurate explanation for the progression of NAFLD. (Huang et al., 2020) NAFLD management's US guidelines define steatosis with (Pouwels et al., 2022) ≥5% fat infiltration in imaging or histology and b) no alcohol, drug, or viral-induced steatosis. Patients may present with elevated liver enzymes and often have one or more components of the metabolic syndrome (MS) like systemic hypertension, dyslipidemia, insulin resistance, or overt diabetes. Visceral obesity is increasingly evidenced as a risk factor for NAFLD, and MS is a known risk factor in cardiovascular disease development (Pouwels et al., 2022). NAFLD is a spectrum of the disease characterized by hepatic steatosis when no other causes for secondary hepatic fat accumulation can be identified. It ranges from the more benign condition of non-alcoholic fatty liver to non-alcoholic steatohepatitis (NASH), which is at the more severe end of the spectrum. NAFLD may progress to fibrosis and cirrhosis (Chalasani et al., 2018). The complicated condition known as non-alcoholic fatty liver disease is impacted by a number of variables, including oxidative stress and other pathogenetic factors. A connection exists between the development of NAFLD and the lipotoxic liver damage caused by fatty acids and their metabolites. Hepatic cells are shielded from ROS damage by an antioxidant defense mechanism, which is impacted by both genetic and epigenetic factors. NAFLD can be effectively treated with a healthy diet and exercise regimen, however patient adherence is poor. Antioxidants like Vitamin E are also employed. Probiotics, prebiotics, nutrition, and faecal microbiota transplantation are emerging therapeutic techniques that target gut microbiota dysbiosis, and natural polyphenols have been suggested as a means of preventing and treating non-alcoholic fatty liver disease. In the future, precision medicine may assist in choosing the optimal course of therapy for a given patient by considering genetic or environmental epigenetic risk factors (Delli Bovi et al., 2021).

Pathophysiology

DNA methylation plays a significant role in the progression of NAFLD and liver fibrosis. These changes affect genes involved in glucose, lipid, or acetyl-coenzyme A metabolism, insulin-like signalling, and mitochondrial function. NASH accelerates epigenetic age by promoting changes in methylation associated with hepatic collagen content. An untargeted evaluation of DNA methylation in liver tissues of patients with NAFLD identified almost 70,000 differentially methylated CpG sites in patients with advanced liver fibrosis (F3-F4) compared to those with no or mild fibrosis (F0-F1). 76% of these sites were hypomethylated and 24% were hypermethylated in advanced liver fibrosis in NAFLD, with 7% of reported methylations correlated with gene expression levels (Kitamoto et al., 2015; Murphy et al., 2013).

DNA methylation is particularly involved in the activation of hepatic stellate cells and their differentiation to myofibroblast, which are crucial procedures for hepatic fibrogenesis. Changes in methylation of specific genes have been linked with these processes, with genes promoting fibrogenesis being hypomethylated and highly expressed, while genes inhibiting hepatic stellate cell activation are hypermethylated and lower expressed in the liver of patients with advanced fibrosis compared to those with mild disease. Hepatic alterations in DNA methylation may be associated with systemic

metabolic outcomes, such as decreased mRNA expression of PPARGC1A, a major regulator of mitochondrial biogenesis, and increased insulin resistance. A recent study focused on differentially methylated regions that form networks associated with the progression of NAFLD, identifying two important networks: one affecting cytoskeleton organization, transcriptional activity, and cell proliferation, and another associated with metabolic pathways (Sookoian et al., 2010).

Histone modifications are also important epigenetic changes that affect transcriptional activity and refer to several posttranslational procedures such as acetylation, phosphorylation, methylation, and ubiquitination. Acetylation status has been most vigorously studied and is considered the net result of histone acetylation by histone acetyltransferases (HATs) and histone deacetylation by histone deacetylases (HDACs), p300, a protein involved in the transcription of carbohydrate-responsive element-binding protein (ChREBP), is linked to the development of non-alcoholic fatty liver disease (J. Lee et al., 2017). Glucose-induced activation of p300 increases the transcription of ChREBP, stimulating lipogenic genes through histone acetylation. Tannic acid attenuates the effects of p300, reducing lipogenesis-related genes and improving NAFLD in mice. Inhibition of cdk4 protein reduces the formation of C/EBPα – p300 complexes, reducing liver steatosis and correcting age-associated liver changes. P300 may also be involved in the activation of hepatic stellate cells and their trans differentiation to myofibroblasts. Sirtuins, particularly Sirtuin 1 (SIRT1), regulate hepatic metabolism and insulin sensitivity. Deficiency of SIRT3 leads to insulin resistance, hyperlipidaemia, and steatohepatitis in mice (J. H. Lee et al., 2014). HDAC3, a member of human class I HDACs, is implicated with circadian metabolic rhythm and deletion leads to hepatic steatosis in mouse liver (Perakakis et al., 2020).

Type 2 diabetes, obesity, and MetS are major global health challenges with significant economic impact. Nonalcoholic fatty liver disease often co-occurs with other metabolic disorders, resulting in multiple health challenges and increased risk of serious clinical consequences. The term NAFLD has been proposed by an international expert panel to describe hepatic steatosis associated with metabolic dysfunction. Patients with NAFLD may be primarily under the care of non-hepatology specialists, and optimal patient care requires effective multidisciplinary collaboration and joint protocols (Lu et al., 2018). A combined approach of pharmacotherapy, lifestyle, and behavioral interventions is likely to be most successful due to the complex nature of metabolic disorders. Proactive assessment and rapid intervention of comorbidities by relevant specialist clinicians is necessary. Building on the chronic disease management approach for patients with T2DM or obesity is possible (N. Tanaka et al., 2019). Our understanding of the natural history and pathogenesis of NAFLD and NASH, focused efforts on new diagnostic and interventional approaches, and ability to deliver optimal multidisciplinary care provide opportunities to improve outcomes and reduce healthcare system impact (Cariou et al., 2021).

Antioxidants used for treatment of Non-alcoholic fatty acid damage / toxicity

Glutathione: Pharmacological aspects and implications for clinical use in non-alcoholic fatty liver disease

Although a few medications are being studied, there is currently no authorized treatment for the common liver illness NAFLD (Powell et al., 2021). Glutathione (GSH) is a tripeptide that is produced in the cytoplasm of cells and may exist in two different forms: reduced and oxidized. Because of its antioxidant properties, there has been conjecture on its potential therapeutic use in long-term conditions such as cancer, chronic liver illnesses, and neurological disorders. One of the most common is non-alcoholic fatty liver disease (NAFLD), which is defined by lipid build-up in hepatocytes without alcohol usage or other steatogenic causes. Hepatic steatosis, often referred to as non-alcoholic fatty liver, and its inflammatory progressive form, non-alcoholic steatohepatitis, which is linked to elevated oxidative stress and reactive oxygen species and ultimately results in liver fibrosis, are included in

the term. While oxidative stress plays a well-established pathogenetic role in many disorders, nothing is known about GSH's potential therapeutic benefit in these illnesses (Santacroce et al., 2023).

Impact of vitamin E on redox biomarkers in non-alcoholic fatty liver disease

In NAFLD pathogenesis, oxidative stress—an imbalance between the formation of reactive species and antioxidant defence—is essential. In order to evaluate the efficacy of therapies aimed at redox imbalances and reactive species, new experimental techniques are required. In NAFLD patients, the immunohistochemical identification of 4-HNE protein adducts has been verified (Podszun et al., 2020). In Western nations, non-alcoholic fatty liver disease is a prevalent liver ailment marked by excessive lipid buildup. Free radical-induced oxidation of macromolecules, especially lipids, appears to be a hallmark of NAFLD and NASH, according to data from human studies. In animal tests and liver biopsies, redox indicators can be affected by vitamin E, especially α-tocopherol. Clinical research indicates that NAFLD and NASH are associated with reactive species-mediated damage to macromolecules, primarily lipids. Patients with NAFLD may experience less oxidative stress if they take at least 200 I.U. of α-tocopherol daily (Podszun & Frank, 2021).

Effects of Oral Vitamin C Supplementation on Liver Health and Associated Parameters in Patients with Non-Alcoholic Fatty Liver Disease: A Randomized Clinical Trial

Nutrient deficiencies and excessive calorie consumption are important dietary risk factors that lead to non-alcoholic fatty liver disease (Chakravarthy et al., 2020). Antioxidant treatment together with lifestyle modifications are commonly used to prevent and cure NAFLD (Romero-Gómez et al., 2017). Due to a big experiment that shown vitamin E improves histological and biochemical aspects in people with non-alcoholic steatohepatitis (NASH) more efficiently than pioglitazone, vitamin E is now frequently suggested as an antioxidant (Sanyal et al., 2010). In primary and secondary preventive studies, vitamin E was found to raise insulin resistance, plasma triacylglycerol levels, and even death rates when given over two years (Bjelakovic et al., 2007; Musso et al., 2012). Compared to fat-soluble vitamin E, vitamin C, a water-soluble vitamin, is surprisingly safe when taken orally, even at 10–10 times the recommended daily limit (Khoshnam-Rad & Khalili, 2019). Prior research indicates that vitamin C in combination with other nutrients, such as vitamin E or resveratrol, may mitigate hepatic steatosis; nevertheless, the effect of vitamin C on liver function is yet unknown (Ivancovsky-Wajcman et al., 2019; Izdebska et al., 2017). Vitamin C is essential for preserving lipid homeostasis in hepatic and circulatory tissues, as evidenced by animal studies (He et al., 2021).

Potential role of inflammation in relation to dietary sodium and β-carotene with non-alcoholic fatty liver disease: A mediation analysis

Dietary salt consumption and non-alcoholic fatty liver disease have been linked independently in earlier research. Higher estimated 24-hour urine salt excretion and non-alcoholic fatty liver disease were shown to be significantly correlated in research utilizing data from the Korea National Health and Nutrition Examination Surveys. Greater dietary salt intake was associated with a higher frequency of non-alcoholic fatty liver disease (NAFLD) in young and middle-aged general adults, according to a Korean research. Greater dietary salt intake was shown to be positively correlated with NAFLD in the PREVEND cohort trial, with an OR per SD increase of 1.30 (95% CI: 1.21–1.41). For HSI-defined NAFLD, comparable outcomes were seen, with a matching OR and 95% CI of 1.40 (1.31–1.51) (Choi et al., 2016; Huh et al., 2015; van den Berg et al., 2019). There is an inverse relationship between dietary β-carotene consumption and non-alcoholic fatty liver disease, whereas dietary salt intake is associated with a higher risk of NAFLD. According to the mediation study, inflammation may be involved in this relationship, since a higher salt intake raises the risk of NAFLD by upregulating inflammation (Chen et al., 2022).

The association between non-alcoholic fatty liver disease and advanced fibrosis with blood selenium level based on the NHANES 2017-2018

There is a strong correlation between blood selenium levels and advanced liver fibrosis/NAFLD. NAFLD was regarded as a metabolic disorder up until that point (Nagy et al., 2016). The greatest risk condition was metabolic syndrome, which is characterized by elevated body mass index, a larger waist circumference, poor fasting glucose, and diabetes (Krausova et al., 2020). Furthermore, sedentary behaviour and inadequate physical activity were found to be independent risk factors for NAFLD in recent research (Khambu et al., 2018; Krausova et al., 2021). Smoking increases the risk of non-alcoholic fatty liver disease in obese rats by increasing insulin resistance, hepatic lipogenesis, and hypercholesterolemia (S. Tanaka et al., 2016). Furthermore, exercise helps lessen the oxidative damage brought on by ROS in NAFLD (G.-L. Song et al., 2018). Selenium has demonstrated a critical involvement in several disorders, particularly those related to metabolism (Y. M. Song et al., 2015). However, further research is still needed to fully understand the connection between selenium and NAFLD. Selenium has typically been acknowledged as an antioxidant that can slow the progression of NAFLD (Sun et al., 2018). By directly substituting high-fat diets (HBD) with more selenomethionine, Yang Yi and Seyedeh et al. discovered that high selenium exposure reduced liver steatosis, HOMA-IR, LDL/HDL-c, and TC/HDL-c ratios—all of which are aetiologies of non-alcoholic fatty liver disease in mouse models. Furthermore, the NAFLD mice model's elevated blood selenium and selenoproteins reduced inflammation, lipogenesis, lipid metabolism dysfunction, and oxidative stress. It also delayed the progression of simple steatosis to NASH, and even liver fibrosis and cirrhosis. Nonetheless, a number of clinical investigations revealed a favourable correlation between high selenium intake and NAFLD (Heo et al., 2016). Thus, we looked at the relationship between blood selenium levels and NAFLD in Americans that was identified by VCTE (Pant et al., 2023). The study found a favourable relationship between blood selenium levels and non-alcoholic fatty liver disease in the US population. Less selenium in the blood indicated a higher proportion of individuals with advanced liver fibrosis. The study found that when it comes to blood selenium, changes in selenium homeostasis rather than dietary selenium consumption are more likely to cause NAFLD and liver fibrosis. This suggests that the root cause of both NAFLD and liver fibrosis is an imbalance in selenium homeostasis (Liu et al., 2022).

Non-alcoholic fatty liver disease: The role of quercetin and its therapeutic implications

Because it can lead to liver problems including cirrhosis and hepatocellular cancer, non-alcoholic fatty liver disease is the most common chronic liver disease, with increased morbidity and death (Rafiei et al., 2017). To develop therapies for liver steatosis prevention, the molecular pathways driving lipid build-up, mitochondrial dysfunction, and increased oxidative stress inside hepatocytes are currently being researched (Dongiovanni et al., 2016). Through regulatory processes such as dietary lipids, lipogenesis, FFA absorption, and VLDL production, the liver maintains an equilibrium between fat input and outflow. Hepatic steatosis results from an excessive build-up of TG in liver cells caused by disruption of this homeostatic mechanism. Reactive oxygen species, fibro genic cytokines, and the recruitment of inflammatory cells are all factors linked to liver fibrosis (Tiniakos et al., 2010). In clinical practice, QE, a naturally occurring molecule with anticancer, anti-inflammatory, and antioxidant properties, has demonstrated encouraging outcomes. It is the flavonoid family's most potent free radical scavenger, preventing both non-alcoholic fatty liver disease and liver steatosis. Depending on its quantity and how it interacts with tissue cells, QE may have prooxidant and proapoptotic effects. It possesses strong antioxidative stress activity and inhibitory effects on hepatocyte apoptosis, inflammation, and ROS formation; its hepatoprotective function is still to be investigated. By lowering CYP2E1 levels and decreasing obesity-induced hepatosteatosis, it shields the liver against NASH. It also increases mitochondrial oxidative metabolism through heme oxygenase-1 and stimulates hepatic mitochondrial

oxidative metabolism through the Nrf-2 pathway (Sotiropoulou et al., 2021).

The effect of turmeric on lipid profile, malondialdehyde, liver echogenicity and enzymes among patients with non-alcoholic fatty liver disease: A randomized double-blind clinical trial

We conducted an RCT of the efficacy of turmeric on some parameters of lipid profile, oxidative stress, liver echogenicity and liver functional test (AST, ALT, and GGT) among NAFLD patients. Overall the results of our study showed that supplementation with turmeric extracts (2000 mg/day) could reduce serum levels of ALT and AST. (Jarhahzadeh et al., 2021) Elevated blood ALT and AST are conventional indicators of liver injury and usually measured in investigations on liver disease (Yam et al., 2007). As mentioned, a combination of insulin resistance, oxidative stress, lipid peroxidation and inflammation are involved in pathogenesis of NAFLD (Angelico et al., 2005; Mavrogiannaki & Migdalis, 2013; Samuel et al., 2004). Hence, any compound that controls all of these disorders could consider as a liver-protective compound. In the current research, supplementation with Turmeric significantly reduced serum levels of AST, ALT, and GGT. These findings were in agreement with two recent systematic reviews and meta-analyses that show the beneficial impact of turmeric and its active component, curcumin supplementation on reduction of serum ALT levels in subgroups with ≥ 1000 mg/day as well as serum levels of AST in studies with 8-weeks administration (Jalali et al., 2020; Mansour-Ghanaei et al., 2019). Moreover, another meta-analysis of 4 randomized controlled trials (RCTs) indicated a considerable effect of the curcumin supplementation on lowering AST levels compared to the placebo; while, there was no significant change in ALT blood concentrations following curcumin consumption (Z. Wei et al., 2019). Supplementation with turmeric extracts reduce elevated serum levels of ALT and AST among patients with NAFLD. Decreasing of these two enzymes could indicate improvement in liver function. Therefore, it could be considered as a good adjuvant therapeutic supplement with hypo lipidemic and antioxidant properties for this disease. However, more well-designed randomized clinical trials are needed to investigate other indicators of NAFLD. Furthermore, the beneficial role of curcumin in other liver diseases remained unclear due to the lack of trials on these populations (Jarhahzadeh et al., 2021).

Efficacy of resveratrol supplementation on liver enzymes in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis

We conducted a systematic review (Darand et al., 2021) to evaluate the effectiveness and safety of resveratrol supplementation for improving liver enzymes in adults with NAFLD. Our results showed that resveratrol cannot effectively reduce AST and ALT concentrations compared with control. Results of subgroup analysis regarding AST revealed that resveratrol supplementation could significantly decrease AST levels in the participants with mean age <45 years, studies with intervention dosage <1000 mg/day, and participants with BMI < 30 kg/m^2. Also, it was observed that resveratrol supplementation significantly decreased the circulating concentrations of ALT levels with duration of follow up >12 weeks. Our findings were in line with recent meta-analysis which examined the effects of resveratrol in adults (Darand et al., 2021). In contrary to our study that only patients with NAFLD were included, they examined subjects with various diseases. Similar results can show that the efficacy of resveratrol is not affected by metabolic status and disease background. Numerous plant species contain the phytoestrogen resveratrol, which has anti-inflammatory and antioxidant qualities. Its anti-inflammatory, anti-aging, cardioprotective, and anti-platelet aggregation qualities have all been demonstrated in studies. Because of these characteristics, resveratrol has an encouraging potential for the management of non-alcoholic fatty liver disease by inhibiting the activity of liver enzymes. It has been shown that supplementing with resveratrol can effectively lower the blood concentrations of hepatic liver enzymes in NAFLD patients, such as ALT and AST (S. Wei & Yu, 2021).

Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats

CP elicits anticancer effects by interacting with DNA and inducing programmed cell death. Multiple in vitro studies have demonstrated the cytotoxic effects of CP in different cell lines, but only a few in vivo studies have been performed (Ahmad et al., 2019; Alhoshani et al., 2017; Karale & Kamath, 2017; Kumburovic et al., 2019; Li et al., 2017; Rjeibi et al., 2018; Zhu et al., 2017). Our findings are consistent with the in vivo results of other studies, including the involvement of oxidative stress and apoptotic mechanisms in CP-induced hepatorenal damage and the potential use of LP and NAC as protective agents against CP-induced injury. Elevated activities of liver enzymes indicate cellular leakage and loss of functional hepatocyte integrity; the liver enzymes are released into the bloodstream when hepatocyte plasma membranes are impaired (Mohamed & Badawy, 2019). In this study, CP-induced hepatotoxicity was evidenced by significant alternations in serum liver enzymes (AST, ALT, and ALP). CP is taken up by the liver and accumulates in hepatocytes, causing cellular damage that eventually leads to increases circulating liver enzymes. In addition, CP elevated creatinine and urea levels, in agreement with previous studies (Abdel-Daim et al., 2019; Abo-Elmaaty et al., 2020; Elkomy et al., 2020). Elevated creatinine and urea levels are caused by reduced glomerular filtration rate. Moreover, the toxicity of the liver and kidney caused by CP to free radicals that generate in the cells of the liver and kidney, resulting in peroxidation of the lipid and consequently leads to oxidative stress that damage cells (Shahid et al., 2018). A derivative of vitamin A, lycopene (LP) possesses anti-inflammatory, immunostimulant, antibacterial, and anti-mutagenic qualities. It can stop the hepatoxicity and nephrotoxicity that come with chemotherapy, which can seriously harm the liver and kidney's tissue because of oxidative stress and apoptotic processes. By combining LP and NAC, one may significantly protect the hepatorenal system against oxidative stress and apoptosis caused by CP-mediated damage to the liver and kidney (Elsayed et al., 2021).

Efficacy and safety of dietary polyphenol supplementation in the treatment of non-alcoholic fatty liver disease

A systematic review and meta-analysis: NAFLD, a liver disease with a prevalence of 20%-30% in the general population and >25% in most Asian countries, is a complex disease regulated by various mechanisms such as glucose and lipid metabolism, genes, environment, and gut microecology (Pierantonelli & Svegliati-Baroni, 2019; Simental-Mendía et al., 2021). Researchers have been exploring the pathogenesis, prevention, and treatment of NAFLD, with the "second hit" hypothesis being widely recognized (Cobbina & Akhlaghi, 2017).This theory suggests that the pathogenesis of NAFLD is closely related to insulin resistance, which is the central link in the occurrence and development of NAFLD. Abnormal insulin signalling pathways and lipid metabolism disorders jointly promote the occurrence and development of NAFLD (Castera et al., 2019; Cotter & Rinella, 2020; Gallego-Duran et al., 2021; Manne et al., 2018). The major sites of P-oxidation of free fatty acids in the liver are mitochondria, microsomes, and peroxisomes. Insulin resistance and hyperinsulinemia promote the release of free fatty acids from peripheral adipose tissue into the liver (Yang et al., 2022), accelerate the utilization of free fatty acids by hepatocytes, and synthesize excess triglycerides in the liver. This leads to abnormal mitochondrial oxidative phosphorylation and lipid P-oxidation, abnormal triglyceride transport, and reduced low-density lipoprotein secretion, resulting in benign liver fat accumulation, called "simple fatty liver." Steatosis is a necessary condition for the development of NAFLD (Polyzos et al., 2019). The meta-analysis indicates that by lowering BMI, TG, TC, liver enzymes, and insulin resistance, polyphenol supplementation may be able to lower the risk of non-alcoholic fatty liver disease. The advantages vary depending on the kind of polyphenol. For example, curcumin (80–3,000 mg, 8–12 weeks) can successfully lower BMI, TG, TC, liver enzymes, and insulin resistance. On the other hand, silymarin (94–2,100 mg, 8–48 weeks) and

catechin (500–1,000 mg, 12 weeks) can also effectively lower liver enzymes. On the other hand, a smaller number of RCTs and no effectiveness were found for certain polyphenols, such as resveratrol, suggesting that further RCTs are required to assess their safety and efficacy (Carr et al., 2016).

Salubrious Effects of Green Tea Catechins on Fatty Liver Disease: A Systematic Review

Many studies have been conducted on the possible health benefits and therapeutic effects of green tea catechins, namely Epigallocatechin-3-gallate (EGCG), in non-alcoholic fatty liver disease. Green tea extract contains anti-inflammatory, antioxidative, and antilipidemic qualities. It is also high in flavonoids. Clinical investigations and animal models have demonstrated the substantial advantages of EGCG. It has also demonstrated favourable effects in type II diabetes, cancer, cardiovascular disease, and metabolic health (Esmaeelpanah et al., 2021). There are a few possibilities in clinical studies, but there are no FDA-approved treatments for NAFLD at this time (Wong & Singal, 2019). Exercise has been demonstrated to stop the advancement of NAFLD and NASH, which makes EGCG and GTE a safe and effective substitute Exercise helps stop NAFLD and NASH from becoming worse, however EGCG and GTE (green tea extract) could be safe substitutes for people who don't have much time or mobility. The liver may be protected against damage and inflammation by these antioxidants. Even while regular exercise has many health advantages that go beyond liver health, EGCG and GTE may have comparable protective effects on the liver, making them useful for people who are unable to exercise regularly. They may be thought of as supplemental or additional therapies to enhance liver function, but they shouldn't be used in place of exercise. Before making any changes to treatment or lifestyle, it is imperative to consult with medical professionals. (Abunofal & Mohan, 2022; Machado, 2021).

Role of N-Acetylcysteine in the Treatment of Acute Nonacetaminophen, Non-alcoholic and Nonviral Hepatitis: A Meta-analysis

This is the first comprehensive evaluation and meta-analysis assessing the efficacy of NAC in treating acute hepatitis brought on by non-viral, non-acetaminophen, and non-alcohol causes. According to the study, NAC had no beneficial effects on hospital stays, infectious complications, or mortality rates in individuals unrelated to acetaminophen, alcohol, or viral infection. As a mucolytic and in cases of acetaminophen overdose, NAC is now licensed for usage (Kolarov & Zvezdin et al., 2022). Since NAC is now the first-line treatment for acetaminophen toxicity and alcoholic hepatitis, NAC not included in the research (Dludla et al., 2020; Jyani et al., 2019). In cases where information is scarce, the research additionally looked at ischemic, post-liver transplant, hypoxia-induced, non-alcoholic, and post-liver transplant hepatitis (Andrade et al., 2019; Garcia-Cortes et al., 2020). NAC was included in the ACG guideline for addressing idiosyncratic drug-induced liver damage because of its favourable safety profile; nevertheless, definitive remedies are not provided (Darweesh et al., 2017). Larger trials are required, although the data do not support the usage of NAC. Although NAC has been investigated in several contexts, this meta-analysis does not support its utility (Aljohani et al., 2021).

Conclusion

The study emphasizes how phytochemicals including green tea catechins, curcumin, resveratrol, quercetin, and silymarin may be able to lessen the liver damage caused by NAFLD. The many antioxidative processes exhibited by these antioxidants impact several pathways, including the activation of nuclear factor-erythroid 2-related factor 2 and the reduction of pro-inflammatory mediators. The results imply that these antioxidants enhance liver function generally in addition to lowering hepatic steatosis. To close current information gaps, standardize dosing schedules, investigate synergistic effects, and comprehend long-term

safety profiles, further study is necessary. It is imperative to adopt a comprehensive strategy that includes dietary treatments, lifestyle adjustments, and antioxidants derived from plants. The knowledge gathered from this study underscores the need to carry out more research into the therapeutic potential of antioxidants derived from plants, as their incorporation into tailored treatment regimens for non-alcoholic fatty liver disease holds potential for long-term, easily obtainable, and integrative medicine-based liver health.

Authors contributions

M.R.M: Contributes to reference management. M.A.N: Contributed to reviewing & editing. All authors have Read & Agreed.

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Conflict of interest

The authors declare no conflict of interest.

References

Abdel-Daim, M. M., Eissa, I. A. M., Abdeen, A., Abdel-Latif, H. M. R., Ismail, M., Dawood, M. A. O., & Hassan, A. M. (2019). Lycopene and resveratrol ameliorate zinc oxide nanoparticles-induced oxidative stress in Nile tilapia, Oreochromis niloticus. *Environmental Toxicology and Pharmacology*, *69*, 44–50.

Abo-Elmaaty, A. M. A., Behairy, A., El-Naseery, N. I., & Abdel-Daim, M. M. (2020). The protective efficacy of vitamin E and cod liver oil against cisplatin-induced acute kidney injury in rats. *Environmental Science and Pollution Research*, *27*, 44412–44426.

Abunofal, O., & Mohan, C. (2022). Salubrious Effects of Green Tea Catechins on Fatty Liver Disease: A Systematic Review. *Medicines*, *9*(3), 20. https://doi.org/10.3390/medicines9030020

Ahmad, S., Hussain, A., Hussain, A., Abdullah, I., Ali, M. S., Froeyen, M., & Mirza, M. U. (2019). Quantification of berberine in Berberis vulgaris L. root extract and its curative and prophylactic role in cisplatin-induced in vivo toxicity and in vitro cytotoxicity. *Antioxidants*, *8*(6), 185.

Alhoshani, A. R., Hafez, M. M., Husain, S., Al-Sheikh, A. M., Alotaibi, M. R., Al Rejaie, S. S., Alshammari, M. A., Almutairi, M. M., & Al-Shabanah, O. A. (2017). Protective effect of rutin supplementation against cisplatin-induced Nephrotoxicity in rats. *BMC Nephrology*, *18*(1), 1–10.

Aljohani, W., Chan, B. P. H., & Yaghoobi, M. (2021). Role of N -Acetylcysteine in the Treatment of Acute Nonacetaminophen, Nonalcoholic and Nonviral Hepatitis: A Meta-analysis. *Journal of the Canadian Association of Gastroenterology*, *4*(3), 125–130. https://doi.org/10.1093/jcag/gwaa017

Andrade, R. J., Chalasani, N., Björnsson, E. S., Suzuki, A., Kullak-Ublick, G. A., Watkins, P. B., Devarbhavi, H., Merz, M., Lucena, M. I., Kaplowitz, N., & Aithal, G. P. (2019). Drug-induced liver injury. *Nature Reviews Disease Primers*, *5*(1), 58. https://doi.org/10.1038/s41572-019-0105-0

Angelico, F., Del Ben, M., Conti, R., Francioso, S., Feole, K., Fiorello, S., Cavallo, M. G., Zalunardo, B., Lirussi, F., & Alessandri, C. (2005). Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. *The Journal of Clinical Endocrinology & Metabolism*, *90*(3), 1578–1582.

Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2007). Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *Jama*, *297*(8), 842–857.

Cariou, B., Byrne, C. D., Loomba, R., & Sanyal, A. J. (2021). Nonalcoholic fatty liver disease as a metabolic disease in humans: A literature review. *Diabetes, Obesity and Metabolism*, *23*(5), 1069–1083.

Carr, R. M., Oranu, A., & Khungar, V. (2016). Nonalcoholic fatty liver disease: pathophysiology and management. *Gastroenterology Clinics*, *45*(4), 639–652.

Castera, L., Friedrich-Rust, M., & Loomba, R. (2019). Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. *Gastroenterology*, *156*(5), 1264–1281.

Chakravarthy, M. V, Waddell, T., Banerjee, R., & Guess, N. (2020). Nutrition and nonalcoholic fatty liver disease: current perspectives. *Gastroenterology Clinics*, *49*(1), 63–94.

Chalasani, N., Younossi, Z., Lavine, J. E., Charlton, M., Cusi, K., Rinella, M., Harrison, S. A., Brunt, E. M., & Sanyal, A. J. (2018). The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*, *67*(1), 328–357.

Chen, Y., Wu, M., Chen, F., Wen, X., Zhao, L., Li, G., & Zhou, L. (2022). Potential role of inflammation in relation to dietary sodium and β-carotene with non-alcoholic fatty liver disease: a mediation analysis. *Nutrition & Diabetes*, *12*(1), 40.

Choi, Y., Lee, J. E., Chang, Y., Kim, M. K., Sung, E., Shin, H., & Ryu, S. (2016). Dietary sodium and potassium intake in relation to non-alcoholic fatty liver disease. *British Journal of Nutrition*, *116*(8), 1447–1456.

Cobbina, E., & Akhlaghi, F. (2017). Non-alcoholic fatty liver disease (NAFLD)–pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. *Drug Metabolism Reviews*, *49*(2), 197–211.

Cotter, T. G., & Rinella, M. (2020). Nonalcoholic fatty liver disease 2020: the state of the disease. *Gastroenterology*, *158*(7), 1851–1864.

Darand, M., Farrokhzad, A., Ghavami, A., Hadi, A., Karimi, E., Fadel, A., & Askari, G. (2021). Effects of resveratrol supplementation on liver enzymes: A systematic review and meta-analysis of randomised controlled trials. *International Journal of Clinical Practice*, *75*(3), e13692.

Darweesh, S. K., Ibrahim, M. F., & El-Tahawy, M. A. (2017). Effect of N-Acetylcysteine on Mortality and Liver Transplantation Rate in Non-Acetaminophen-Induced Acute Liver Failure: A Multicenter Study. *Clinical Drug Investigation*, *37*(5), 473–482. https://doi.org/10.1007/s40261-017-0505-4

Delli Bovi, A. P., Marciano, F., Mandato, C., Siano, M. A., Savoia, M., & Vajro, P. (2021). Oxidative stress in non-alcoholic fatty liver disease. An updated mini review. *Frontiers in Medicine*, *8*, 165.

Dludla, P. V., Nkambule, B. B., Mazibuko-Mbeje, S. E., Nyambuya, T. M., Marcheggiani, F., Cirilli, I., Ziqubu, K., Shabalala, S. C., Johnson, R., Louw, J., Damiani, E., & Tiano, L. (2020). N-Acetyl Cysteine Targets Hepatic Lipid Accumulation to Curb Oxidative Stress and Inflammation in NAFLD: A Comprehensive Analysis of the Literature. *Antioxidants*, *9*(12), 1283. https://doi.org/10.3390/antiox9121283

Dongiovanni, P., Lanti, C., Riso, P., & Valenti, L. (2016). Nutritional therapy for nonalcoholic fatty liver disease. *The Journal of Nutritional Biochemistry*, *29*, 1–11.

Elkomy, A., Abdelhiee, E. Y., Fadl, S. E., Emam, M. A., Gad, F. A.-M., Sallam, A., Alarifi, S.,

Abdel-Daim, M. M., & Aboubakr, M. (2020). L-carnitine mitigates oxidative stress and disorganization of cytoskeleton intermediate filaments in cisplatin-induced hepato-renal toxicity in rats. *Frontiers in Pharmacology*, *11*, 574441.

Elsayed, A., Elkomy, A., Elkammar, R., Youssef, G., Abdelhiee, E. Y., Abdo, W., Fadl, S. E., Soliman, A., & Aboubakr, M. (2021). Synergistic protective effects of lycopene and N-acetylcysteine against cisplatin-induced hepatorenal toxicity in rats. *Scientific Reports*, *11*(1), 13979.

Esmaeelpanah, E., Razavi, B. M., & Hosseinzadeh, H. (2021). Green tea and metabolic syndrome: A 10-year research update review. *Iranian Journal of Basic Medical Sciences*, *24*(9), 1159–1172. https://doi.org/10.22038/IJBMS.2021.52980.11943

Gallego-Duran, R., Montero-Vallejo, R., Maya-Miles, D., Lucena, A., Martin, F., Ampuero, J., & Romero-Gomez, M. (2021). Analysis of common pathways and markers from non-alcoholic fatty liver disease to immune-mediated diseases. *Frontiers in Immunology*, *12*, 667354.

Garcia-Cortes, M., Robles-Diaz, M., Stephens, C., Ortega-Alonso, A., Lucena, M. I., & Andrade, R. J. (2020). Drug induced liver injury: an update. *Archives of Toxicology*, *94*(10), 3381–3407. https://doi.org/10.1007/s00204-020-02885-1

He, Z., Li, X., Yang, H., Wu, P., Wang, S., Cao, D., Guo, X., Xu, Z., Gao, J., & Zhang, W. (2021). Effects of oral vitamin C supplementation on liver health and associated parameters in patients with non-alcoholic fatty liver disease: a randomized clinical trial. *Frontiers in Nutrition*, *8*, 745609.

Heo, J., Seo, M., Park, H., Lee, W. K., Guan, L. L., Yoon, J., Caetano-Anolles, K., Ahn, H., Kim, S.-Y., Kang, Y.-M., Cho, S., & Kim, H. (2016). Gut microbiota Modulated by Probiotics and Garcinia cambogia Extract Correlate with Weight Gain and Adipocyte Sizes in High Fat-Fed Mice. *Scientific Reports*, *6*(1), 33566. https://doi.org/10.1038/srep33566

Huang, T., Behary, J., & Zekry, A. (2020). Non-alcoholic fatty liver disease: a review of epidemiology, risk factors, diagnosis and management. *Internal Medicine Journal*, *50*(9), 1038–1047.

Huh, J. H., Lee, K. J., Lim, J. S., Lee, M. Y., Park, H. J., Kim, M. Y., Kim, J. W., Chung, C. H., Shin, J. Y., & Kim, H.-S. (2015). High dietary sodium intake assessed by estimated 24-h urinary sodium excretion is associated with NAFLD and hepatic fibrosis. *PloS One*, *10*(11), e0143222.

Ivancovsky-Wajcman, D., Fliss-Isakov, N., Salomone, F., Webb, M., Shibolet, O., Kariv, R., & Zelber-Sagi, S. (2019). Dietary vitamin E and C intake is inversely associated with the severity of nonalcoholic fatty liver disease. *Digestive and Liver Disease*, *51*(12), 1698–1705.

Izdebska, M., Piątkowska-Chmiel, I., Korolczuk, A., Herbet, M., Gawrońska-Grzywacz, M., Gieroba, R., Sysa, M., Czajkowska-Bania, K., Cygal, M., & Korga, A. (2017). The beneficial effects of resveratrol on steatosis and mitochondrial oxidative stress in HepG2 cells. *Canadian Journal of Physiology and Pharmacology*, *95*(12), 1442–1453.

Jalali, M., Mahmoodi, M., Mosallanezhad, Z., Jalali, R., Imanieh, M. H., & Moosavian, S. P. (2020). The effects of curcumin supplementation on liver function, metabolic profile and body composition in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis of randomized controlled trials. *Complementary Therapies in Medicine*, *48*,

102283.

Jarhahzadeh, M., Alavinejad, P., Farsi, F., Husain, D., & Rezazadeh, A. (2021). The effect of turmeric on lipid profile, malondialdehyde, liver echogenicity and enzymes among patients with nonalcoholic fatty liver disease: a randomized double blind clinical trial. *Diabetology & Metabolic Syndrome*, *13*, 1–9.

Jyani, G., Prinja, S., Ambekar, A., Bahuguna, P., & Kumar, R. (2019). Health impact and economic burden of alcohol consumption in India. *International Journal of Drug Policy*, *69*, 34–42. https://doi.org/10.1016/j.drugpo.2019.04.005

Karale, S., & Kamath, J. V. (2017). Effect of daidzein on cisplatin-induced hematotoxicity and hepatotoxicity in experimental rats. *Indian Journal of Pharmacology*, *49*(1), 49.

Khambu, B., Yan, S., Huda, N., Liu, G., & Yin, X.-M. (2018). Autophagy in non-alcoholic fatty liver disease and alcoholic liver disease. *Liver Research*, *2*(3), 112–119. https://doi.org/10.1016/j.livres.2018.09.004

Khoshnam-Rad, N., & Khalili, H. (2019). Safety of vitamin C in sepsis: a neglected topic. *Current Opinion in Critical Care*, *25*(4), 329–333.

Kitamoto, T., Kitamoto, A., Ogawa, Y., Honda, Y., Imajo, K., Saito, S., Yoneda, M., Nakamura, T., Nakajima, A., & Hotta, K. (2015). Targeted-bisulfite sequence analysis of the methylation of CpG islands in genes encoding PNPLA3, SAMM50, and PARVB of patients with non-alcoholic fatty liver disease. *Journal of Hepatology*, *63*(2), 494–502.

Kolarov, V., Kotur-Stevuljević, J., Ilić, M., Bogdan, M., Tušek, B., Agic, A., Dugajlić, M., Tot Vereš, K., Kutlešić Stević, S.,& Zvezdin, B. (2022). Factorial analysis of N-acetylcysteine and propolis treatment effects on symptoms, life quality and exacerbations in patients with Chronic Obstructive Pulmonary Disease (COPD): a randomized, double-blind, placebo-controlled trial. *European Review for Medical and Pharmacological Sciences Verduci Editore Srl.*, *26*,(9,), 3192–3199. https://doi.org/10.26355/eurrev_202205_28737

Krausova, G., Kana, A., Hyrslova, I., Mrvikova, I., & Kavkova, M. (2020). Development of Selenized Lactic Acid Bacteria and their Selenium Bioaccummulation Capacity. *Fermentation 2020, Vol. 6, Page 91*, *6*(3), 91. https://doi.org/10.3390/FERMENTATION6030091

Krausova, G., Kana, A., Vecka, M., Hyrslova, I., Stankova, B., Kantorova, V., Mrvikova, I., Huttl, M., & Malinska, H. (2021). In Vivo Bioavailability of Selenium in Selenium-Enriched Streptococcus thermophilus and Enterococcus faecium in CD IGS Rats. *Antioxidants*, *10*(3), 463. https://doi.org/10.3390/antiox10030463

Kumburovic, I., Selakovic, D., Juric, T., Jovicic, N., Mihailovic, V., Stankovic, J. K., Sreckovic, N., Kumburovic, D., Jakovljevic, V., & Rosic, G. (2019). Antioxidant effects of Satureja hortensis L. attenuate the anxiogenic effect of cisplatin in rats. *Oxidative Medicine and Cellular Longevity*, *2019*.

Lee, J. H., Friso, S., & Choi, S.-W. (2014). Epigenetic mechanisms underlying the link between non-alcoholic fatty liver diseases and nutrition. *Nutrients*, *6*(8), 3303–3325.

Lee, J., Kim, Y., Friso, S., & Choi, S.-W. (2017). Epigenetics in non-alcoholic fatty liver disease. *Molecular Aspects of Medicine*, *54*,

78–88.

Li, C.-Y., Song, H.-T., Wang, X.-X., Wan, Y.-Y., Ding, X.-S., Liu, S.-J., Dai, G.-L., Liu, Y.-H., & Ju, W.-Z. (2017). Urinary metabolomics reveals the therapeutic effect of HuangQi Injections in cisplatin-induced nephrotoxic rats. *Scientific Reports*, *7*(1), 3619.

Liu, J., Tan, L., Liu, Z., & Shi, R. (2022). The association between non-alcoholic fatty liver disease (NAFLD) and advanced fibrosis with blood selenium level based on the NHANES 2017-2018. *Annals of Medicine*, *54*(1), 2258–2267. https://doi.org/10.1080/07853890.2022.2110277

Lu, F.-B., Hu, E.-D., Xu, L.-M., Chen, L. U., Wu, J.-L., Li, H., Chen, D.-Z., & Chen, Y.-P. (2018). The relationship between obesity and the severity of non-alcoholic fatty liver disease: systematic review and meta-analysis. *Expert Review of Gastroenterology & Hepatology*, *12*(5), 491–502.

Machado, M. V. (2021). Aerobic Exercise in the Management of Metabolic Dysfunction Associated Fatty Liver Disease. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, *Volume 14*, 3627–3645. https://doi.org/10.2147/DMSO.S304357

Manne, V., Handa, P., & Kowdley, K. V. (2018). Pathophysiology of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Clinics in Liver Disease*, *22*(1), 23–37.

Mansour-Ghanaei, F., Pourmasoumi, M., Hadi, A., & Joukar, F. (2019). Efficacy of curcumin/turmeric on liver enzymes in patients with non-alcoholic fatty liver disease: a systematic review of randomized controlled trials. *Integrative Medicine Research*, *8*(1), 57–61.

Mavrogiannaki, A. N., & Migdalis, I. N. (2013). Nonalcoholic fatty liver disease, diabetes mellitus and cardiovascular disease: newer data. *International Journal of Endocrinology*, *2013*.

Mohamed, H. E., & Badawy, M. M. M. (2019). Modulatory effect of zingerone against cisplatin or γ-irradiation induced hepatotoxicity by molecular targeting regulation. *Applied Radiation and Isotopes*, *154*, 108891.

Murphy, S. K., Yang, H., Moylan, C. A., Pang, H., Dellinger, A., Abdelmalek, M. F., Garrett, M. E., Ashley–Koch, A., Suzuki, A., & Tillmann, H. L. (2013). Relationship between methylome and transcriptome in patients with nonalcoholic fatty liver disease. *Gastroenterology*, *145*(5), 1076–1087.

Musso, G., Cassader, M., Rosina, F., & Gambino, R. (2012). Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia*, *55*, 885–904.

Nagy, G., Pinczes, G., Pinter, G., Pocsi, I., Prokisch, J., & Banfalvi, G. (2016). In situ electron microscopy of lactomicroselenium particles in probiotic bacteria. *International Journal of Molecular Sciences*, *17*(7), 1047.

Pant, R., Sharma, N., Kabeer, S. W., Sharma, S., & Tikoo, K. (2023). Selenium-Enriched Probiotic Alleviates Western Diet-Induced Non-alcoholic Fatty Liver Disease in Rats via Modulation of Autophagy Through AMPK/SIRT-1 Pathway. *Biological Trace Element Research*, *201*(3), 1344–1357. https://doi.org/10.1007/s12011-022-03247-x

Perakakis, N., Stefanakis, K., & Mantzoros, C. S. (2020). The role of omics in the pathophysiology, diagnosis and treatment of non-alcoholic fatty liver disease.

Metabolism, *111*, 154320. https://doi.org/10.1016/j.metabol.2020.154320

Pierantonelli, I., & Svegliati-Baroni, G. (2019). Nonalcoholic fatty liver disease: basic pathogenetic mechanisms in the progression from NAFLD to NASH. *Transplantation*, *103*(1), e1–e13.

Podszun, M. C., Chung, J.-Y., Ylaya, K., Kleiner, D. E., Hewitt, S. M., & Rotman, Y. (2020). 4-HNE immunohistochemistry and image analysis for detection of lipid peroxidation in human liver samples using vitamin E treatment in NAFLD as a proof of concept. *Journal of Histochemistry & Cytochemistry*, *68*(9), 635–643.

Podszun, M. C., & Frank, J. (2021). Impact of vitamin E on redox biomarkers in non-alcoholic fatty liver disease. *Redox Biology*, *42*, 101937.

Polyzos, S. A., Kountouras, J., & Mantzoros, C. S. (2019). Obesity and nonalcoholic fatty liver disease: From pathophysiology to therapeutics. *Metabolism*, *92*, 82–97.

Pouwels, S., Sakran, N., Graham, Y., Leal, A., Pintar, T., Yang, W., Kassir, R., Singhal, R., Mahawar, K., & Ramnarain, D. (2022). Non-alcoholic fatty liver disease (NAFLD): a review of pathophysiology, clinical management and effects of weight loss. *BMC Endocrine Disorders*, *22*(1), 1–9.

Powell, E. E., Wong, V. W.-S., & Rinella, M. (2021). Non-alcoholic fatty liver disease. *The Lancet*, *397*(10290), 2212–2224.

Rafiei, H., Omidian, K., & Bandy, B. (2017). Comparison of dietary polyphenols for protection against molecular mechanisms underlying nonalcoholic fatty liver disease in a cell model of steatosis. *Molecular Nutrition & Food Research*, *61*(9), 1600781. https://doi.org/10.1002/mnfr.201600781

Rjeibi, I., Feriani, A., Ben Saad, A., Sdayria, J., Saidi, I., Ncib, S., Souid, S., Allagui, M. S., & Hfaiedh, N. (2018). Lycium europaeum extract: a new potential antioxidant source against cisplatin-induced liver and kidney injuries in mice. *Oxidative Medicine and Cellular Longevity*, *2018*.

Romero-Gómez, M., Zelber-Sagi, S., & Trenell, M. (2017). Treatment of NAFLD with diet, physical activity and exercise. *Journal of Hepatology*, *67*(4), 829–846.

Samuel, V. T., Liu, Z.-X., Qu, X., Elder, B. D., Bilz, S., Befroy, D., Romanelli, A. J., & Shulman, G. I. (2004). Mechanism of hepatic insulin resistance in non-alcoholic fatty liver disease. *Journal of Biological Chemistry*, *279*(31), 32345–32353.

Santacroce, G., Gentile, A., Soriano, S., Novelli, A., Lenti, M. V., & Di Sabatino, A. (2023). Glutathione: Pharmacological aspects and implications for clinical use in non-alcoholic fatty liver disease. *Frontiers in Medicine*, *10*, 1124275.

Sanyal, A. J., Chalasani, N., Kowdley, K. V, McCullough, A., Diehl, A. M., Bass, N. M., Neuschwander-Tetri, B. A., Lavine, J. E., Tonascia, J., & Unalp, A. (2010). Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *New England Journal of Medicine*, *362*(18), 1675–1685.

Shahid, F., Farooqui, Z., & Khan, F. (2018). Cisplatin-induced gastrointestinal toxicity: An update on possible mechanisms and on available gastroprotective strategies. *European Journal of Pharmacology*, *827*, 49–57.

Simental-Mendía, L. E., Gamboa-Gómez, C. I., Guerrero-Romero, F., Simental-Mendía, M., Sánchez-García, A., & Rodríguez-Ramírez, M. (2021). Beneficial effects of plant-derived natural products on non-alcoholic fatty liver disease.

Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health, 257–272.

Song, G.-L., Chen, C., Wu, Q.-Y., Zhang, Z.-H., Zheng, R., Chen, Y., Jia, S.-Z., & Ni, J.-Z. (2018). Selenium-enriched yeast inhibited β-amyloid production and modulated autophagy in a triple transgenic mouse model of Alzheimer's disease. *Metallomics*, *10*(8), 1107–1115. https://doi.org/10.1039/C8MT00041G

Song, Y. M., Lee, Y., Kim, J.-W., Ham, D.-S., Kang, E.-S., Cha, B. S., Lee, H. C., & Lee, B.-W. (2015). Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. *Autophagy*, *11*(1), 46–59. https://doi.org/10.4161/15548627.2014.984271

Sookoian, S., Rosselli, M. S., Gemma, C., Burgueño, A. L., Fernández Gianotti, T., Castaño, G. O., & Pirola, C. J. (2010). Epigenetic regulation of insulin resistance in nonalcoholic fatty liver disease: Impact of liver methylation of the peroxisome proliferator–activated receptor γ coactivator 1α promoter. *Hepatology*, *52*(6), 1992–2000.

Sotiropoulou, M., Katsaros, I., Vailas, M., Lidoriki, I., Papatheodoridis, G. V, Kostomitsopoulos, N. G., Valsami, G., Tsaroucha, A., & Schizas, D. (2021). Nonalcoholic fatty liver disease: The role of quercetin and its therapeutic implications. *Saudi Journal of Gastroenterology: Official Journal of the Saudi Gastroenterology Association*, *27*(6), 319.

Sun, Y., Xia, M., Yan, H., Han, Y., Zhang, F., Hu, Z., Cui, A., Ma, F., Liu, Z., Gong, Q., Chen, X., Gao, J., Bian, H., Tan, Y., Li, Y., & Gao, X. (2018). Berberine attenuates hepatic steatosis and enhances energy expenditure in mice by inducing autophagy and fibroblast growth factor 21. *British Journal of Pharmacology*, *175*(2), 374–387. https://doi.org/10.1111/bph.14079

Tanaka, N., Kimura, T., Fujimori, N., Nagaya, T., Komatsu, M., & Tanaka, E. (2019). Current status, problems, and perspectives of non-alcoholic fatty liver disease research. *World Journal of Gastroenterology*, *25*(2), 163.

Tanaka, S., Hikita, H., Tatsumi, T., Sakamori, R., Nozaki, Y., Sakane, S., Shiode, Y., Nakabori, T., Saito, Y., Hiramatsu, N., Tabata, K., Kawabata, T., Hamasaki, M., Eguchi, H., Nagano, H., Yoshimori, T., & Takehara, T. (2016). Rubicon inhibits autophagy and accelerates hepatocyte apoptosis and lipid accumulation in nonalcoholic fatty liver disease in mice. *Hepatology*, *64*(6), 1994–2014. https://doi.org/10.1002/hep.28820

Tiniakos, D. G., Vos, M. B., & Brunt, E. M. (2010). Nonalcoholic fatty liver disease: pathology and pathogenesis. *Annual Review of Pathology: Mechanisms of Disease*, *5*, 145–171.

van den Berg, E. H., Gruppen, E. G., Blokzijl, H., Bakker, S. J. L., & Dullaart, R. P. F. (2019). Higher sodium intake assessed by 24 hour urinary sodium excretion is associated with non-alcoholic fatty liver disease: the PREVEND cohort study. *Journal of Clinical Medicine*, *8*(12), 2157.

Wei, S., & Yu, X. (2021). Efficacy of resveratrol supplementation on liver enzymes in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis. *Complementary Therapies in Medicine*, *57*, 102635.

Wei, Z., Liu, N., Tantai, X., Xing, X., Xiao, C., Chen, L., & Wang, J. (2019). The effects of curcumin on the metabolic parameters of

non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Hepatology International*, *13*, 302–313.

Wong, V. W.-S., & Singal, A. K. (2019). Emerging medical therapies for non-alcoholic fatty liver disease and for alcoholic hepatitis. *Translational Gastroenterology and Hepatology*, *4*, 53. https://doi.org/10.21037/tgh.2019.06.06

Yam, M. F., Basir, R., Asmawi, M. Z., & Ismail, Z. (2007). Antioxidant and hepatoprotective effects of Orthosiphon stamineus Benth. standardized extract. *The American Journal of Chinese Medicine*, *35*(01), 115–126.

Yang, K., Chen, J., Zhang, T., Yuan, X., Ge, A., Wang, S., Xu, H., Zeng, L., & Ge, J. (2022). Efficacy and safety of dietary polyphenol supplementation in the treatment of non-alcoholic fatty liver disease: a systematic review and meta-analysis. *Frontiers in Immunology*, *13*, 949746.

Zhu, X., Jiang, X., Li, A., Zhao, Z., & Li, S. (2017). S-Allylmercaptocysteine attenuates cisplatin-induced nephrotoxicity through suppression of apoptosis, oxidative stress, and inflammation. *Nutrients*, *9*(2), 166.

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Updates on Behavioral Interventions for Smoking Cessation: A Systematic Review of Systematic Reviews

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Abstract

Introduction: In Malaysia, tobacco smoking is one of the leading causes of early and preventable mortality. The 'Clinical Practice Guidelines on Treatment of Tobacco Use Disorder 2016' is utilised to provide safe and effective smoking cessation services for smokers to quit successfully. Since the launch of the 2016 CPG, there have been several new pieces of evidence regarding behavioural interventions for tobacco smoking cessation with various outcomes. Therefore, the guidelines are expected to be updated to assist healthcare providers in helping smokers quit smoking. **Objectives**: This study aims to review the evidence from 2016 onwards for behavioural interventions in smoking cessation reported from published systematic reviews, and to update the CPG on tobacco use disorder by conducting a systematic review of systematic reviews methodology. **Methodology**: The Cochrane Library, PubMed, and Scopus databases were used to conduct a comprehensive literature search. Two reviewers performed the screening and study selection, with disagreements resolved by consensus or the involvement of another reviewer. Quality assessment and data extraction are performed by one reviewer and checked by another. AMSTAR-2 tool was used to perform the risk of bias assessment. A narrative synthesis of the data extracted was provided. **Result**: The searches resulted in a total of 276 articles and out of these, 23 systematic reviews were included. The included studies incorporated various smoking cessation interventions. Smokers of all ages and a small proportion of recent quitters are involved. They may be from the general or the special population. 14 reviews were rated as high quality, 2 were moderate, 4 were low and 3 were critically low by the AMSTAR-2 tool. The analysis found that counselling sessions, online interventions, self-help materials and motivational interviewing may increase cessation rates in the long term, if not, short term. Counselling sessions demonstrate the strongest evidence of benefit in smokers trying to quit. **Conclusion**: Findings that can be added to the updated CPG include app-based, incentives, feedback on spirometry results, exercise and behavioural interventions for people living with HIV and AIDS, COPD patients, and underprivileged older smokers.

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Introduction

Tobacco smoking is one of the biggest public health threats worldwide. Around 7 million died yearly from their smoking habit, while another 1.2 million perished from second-hand smoke exposure (WHO, 2022). In Malaysia, tobacco smoking is considered to be one of the leading causes of early and preventable mortality, with an estimated 20,000 deaths each year (MOH Malaysia, 2016). Long-term smoking causes cancer, heart disease, stroke, lung diseases, diabetes, and chronic obstructive pulmonary disease (COPD), which includes emphysema and chronic bronchitis. Smoking also increases the risk of tuberculosis, certain eye diseases, and immune system problems, including rheumatoid arthritis (CDC, 2020). Most smokers are aware of the dangers of cigarette smoking and desire to quit. However, they may face difficulties, especially those attempting to quit without professional help.

In Malaysia, the 'Clinical Practice Guidelines (CPG) on Treatment of Tobacco Use Disorder 2016' by the Ministry of Health (MOH) Malaysia is utilised to provide safe and effective smoking cessation services for smokers to quit successfully. Tobacco use interventions consist of pharmacological or/and non-pharmacological (behavioural) interventions. This systematic review will only focus on behavioural interventions to assist smokers in quitting. Behavioural interventions are "interventions designed to affect the actions that individuals take concerning their health" as defined by Cutler (2004). Some behavioural interventions discussed in the 2016 CPG include individual or group counselling, quitlines, and online smoking cessation interventions (e.g., text messages, mobile phones, and web-based programmes). Counselling and quitlines aim to strengthen a person's ability to implement their plans to quit smoking and support their motivation to resist smoking. Online smoking cessation interventions delivered via text messaging or the internet show potential for helping smokers to quit because they can reach a large number of smokers and also low cost for the user (MOH Malaysia, 2016).

Since the launch of the 2016 CPG, there have been several new pieces of evidence on behavioural interventions for tobacco smoking cessation with various outcomes. Some behavioural interventions that are not discussed in the 2016 CPG include providing feedback on spirometry results (Westerdahl et al., 2019), incentives (Notley et al., 2019) and exercise-based interventions (Ussher et al., 2019) for smoking cessation. Therefore, the guidelines are expected to be updated to assist healthcare providers in helping smokers quit smoking.

The objective of this systematic review is to review the evidence for behavioural interventions in smoking cessation reported from published systematic reviews. Another objective is to update the clinical practice guidelines on tobacco use disorder by conducting a systematic review of systematic reviews methodology.

Materials and methods

Materials

This systematic review is done according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement guidelines (Page et al., 2021). The methodology in this systematic review includes developing inclusion criteria, designing a search strategy, searching through databases, articles screening and selection, quality assessment, data extraction and results presentation (V. Smith et al., 2011; Tawfik et al., 2019).

Eligibility Criteria

Types of studies

Full-text English systematic reviews published from January 2016 until November 2022 in online databases or peer-reviewed journals. Only systematic reviews with retrievable full-text articles are included. Primary studies, systematic reviews published in languages other than English or reviews that did not clearly define the behavioural intervention and/or its outcome measures are excluded.

Type of participants

Smokers from both general and special populations (e.g., pregnant women, psychiatric patients) who are willing to participate in the intervention. There are no restrictions on the participant's age, gender, or race.

Type of interventions

Any behavioural intervention for quitting conventional cigarette smoking; whether it is provided to individuals or groups, delivered face-to-face or online or both, tailored or not, regardless of the intervention provider or setting.

Type of comparators

Any comparator for the behavioural intervention may be - no intervention, another behavioural intervention type, usual or standard care, or different intensity of the intervention.

Type of outcome measures

Smoking abstinence is assessed at least 6 months from the start of intervention or quit date. Systematic reviews that emphasise other outcomes (e.g., smoking reduction) or do not specify the outcomes are excluded.

Search Strategy

A comprehensive literature search was conducted in the Cochrane Library, PubMed and Scopus databases. Key terms related to behavioural interventions (e.g., 'behavioural therapies', 'behavioural treatments', 'counselling'), and smoking cessation (e.g., 'tobacco cessation', 'tobacco smoking cessation', 'quit smoking') were combined using Boolean Operators. Filter for English language, type of study (systematic reviews) and the publication year (January 2016 to November 2022) is applied. The key terms for the search strategy are listed in **Appendix 1**.

Screening and Selection

Once articles were identified, duplicates were removed by the Mendeley software and manually. Two reviewers (NS, AN) screened the title and abstract of the identified articles. In the screening phase, publications other than systematic reviews will be excluded. The same two reviewers (NS, AN) also reviewed the full texts for eligibility. For inclusion, the systematic reviews must meet all the eligibility criteria mentioned previously. Disagreements are settled by discussion and, if necessary, a third reviewer (NA) is involved. After the full-text review, the systematic reviews remaining are included.

Assessment of Methodological Quality

AMSTAR-2 tool was used to assess the quality of methodology from the eligible systematic reviews (Shea et al., 2017). A reviewer (NS) conducted the

assessment, and another reviewer (AN) checked the result of the assessment. The results for all AMSTAR-2 items and the overall ratings for each eligible review are tabulated.

Data Extraction & Synthesis

A reviewer (NS) performed data extraction, and another reviewer (AN) cross-checked the data extracted. An Excel spreadsheet was used to record all the extracted data. The data include the publication details (e.g., authors, year of publication), population characteristics (e.g., adult smokers), type of behavioural interventions, details regarding

comparator (e.g., no intervention, other behavioural intervention), the outcome of interest, and study findings. The data were tabulated, and a narrative synthesis of the data was provided.

Results

Search Outcomes

PRISMA 2020 flow diagram **(Figure 1)** is used to visualise the screening findings and the selection of eligible reviews (Page et al., 2021). 246 studies were identified and 185 references were excluded based on titles and abstracts, resulting in 61 full-text to retrieve. However, only 57 studies can be retrieved and assessed for eligibility, then another 34 articles were excluded for not meeting the criteria hence the remaining 23 studies are included in the review.

Study Characteristics

Study populations are very diverse. Tobacco smokers of all ages and a small proportion of recent quitters were included. The smokers are either from the general or special population. In most studies, the outcome measure is smoking abstinence, assessed at a minimum of 6 months from the start of the intervention. However, some studies also assessed short-term abstinence (< 6 months). As for pregnant smokers, smoking cessation is assessed at the longest follow-up. **Table 1** summarises the study characteristics of the included review.

The included studies incorporated various behavioural smoking cessation interventions such as mindfulness (Jackson et al., 2022), incentives (Notley et al., 2019), competitions (Fanshawe et al., 2019), telephone counselling (Matkin et al., 2019), real-time video counselling (Tzelepis et al., 2019), individual counselling (Lancaster & Stead, 2017), group therapy (Stead et al., 2017), motivational interviewing (Lindson et al., 2019), print-based self-help materials (Hartmann-Boyce et al., 2014), mobile phone text messaging (Whittaker et al., 2019), app-based

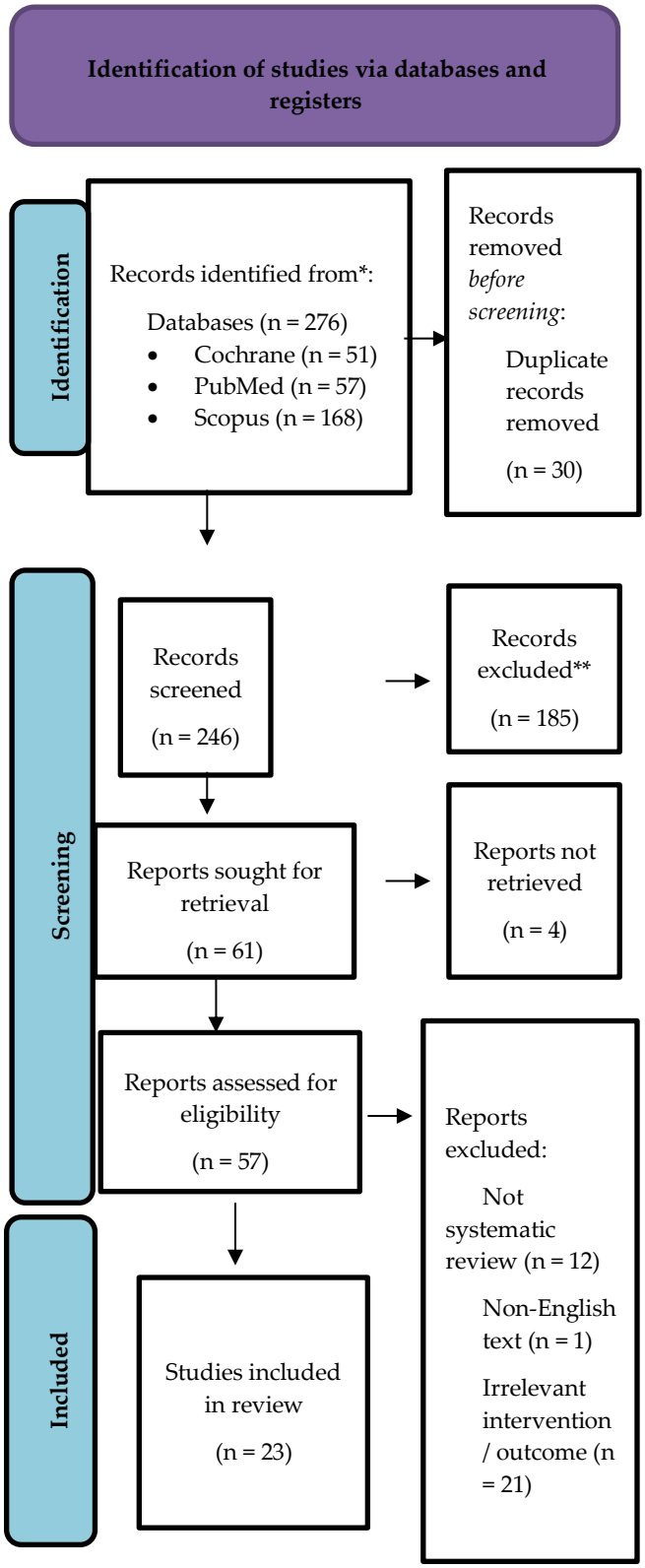

Fig.1. PRISMA 2020 diagram of included studies

intervention (Barroso-Hurtado et al., 2021; Whittaker et al., 2019), internet- or web-based intervention (Do et al., 2018; Taylor et al., 2017)

, feedback on spirometry results (Westerdahl et al., 2019), exercise (Ussher et al., 2019), and hypnotherapy (Barnes et al., 2019).

7 studies focus on providing behavioural interventions for special populations which include pregnant women (Chamberlain et al., 2017), people with severe mental illness (SMI) (Hawes et al., 2021; Spanakis et al., 2022), people living with HIV and AIDS (PLWHA) (Pool et al., 2016), people with chronic obstructive pulmonary disease (COPD) (van Eerd et al., 2016), underprivileged older smokers (Smith et al., 2019), and smokers with substance use disorder (SUD) (Thurgood et al., 2016).

Quality Assessment

The quality assessment for each eligible review is summarized in **Table 2**.

Discussions

A quitter is defined as a smoker who has successfully quit smoking or been abstinent without even a single puff of a cigarette for at least six months from the last cigarette. Six months is a typical period for measuring successful smoking cessation (MOH Malaysia, 2016). Experts also reached a consensus that prolonged or continuous abstinence for at least 6 months is important to measure smoking cessation (Cheung et al., 2017). Hence, this review focuses on behavioural interventions with smoking abstinence for at least six months. The 23 studies included in this review were very heterogeneous in terms of treatment given, comparison groups and outcome measurement; thus, a meta-analysis could not be conducted. The first part of the discussion will review the evidence on the effectiveness of available interventions for smoking cessation.

According to Lancaster & Stead (2017), there is high-quality evidence that individual counselling helps increase the cessation rate with or without pharmacotherapy after at least 6 months. The RR for individual counselling versus non-active controls (i.e., brief advice or self-help materials) was 1.57 (95% CI 1.40 to 1.77) in smokers not receiving pharmacotherapy, and 1.24 (95% CI 1.01 to 1.51) in smokers receiving pharmacotherapy. More intensive counselling moderately has a small relative benefit compared to a brief counselling session. The RR for more intensive versus less intensive counselling was 1.29 (95% CI 1.09 to 1.53).

Stead et al. (2017) found that there is reasonable evidence that group counselling is better in helping people stop smoking than non-active controls. The RR for group therapy versus self-help materials was 1.88 (95% CI 1.52 to. 2.33) while the RR for group therapy versus brief advice was 1.25 (95% CI 1.07 to 1.46). The study also found that there is no evidence that group counselling is superior to intensive individual counselling, whether or not the number of sessions was matched. The RR for group therapy versus individual counselling was 0.99 (95% CI 0.76 to 1.28). Most of the counselling sessions, whether individual or group, included repeated contact but it differs whether face-to-face or telephone contact was used after the initial meeting. However, studies suggest that it may not be important which contact is maintained.

Table 1. Study characteristics summary.

Authors (Publication year)	Number of studies included	PICO				Findings
		Participants	Interventions	Comparator	Outcome measure	
Lancaster & Stead (2017)	49	Any smokers.	Face-to-face encounter between a smoker and a counsellor trained in assisting smoking cessation.	No interventions, non-active interventions (i.e. brief advice or self-help), different counselling intensity.	Smoking cessation at the longest reported follow-up (6 months or more).	There is high-quality evidence that individual counselling can assist smokers in quitting. There is consistent evidence that individual counselling increases the likelihood of cessation compared to less intensive support. There is moderate-quality evidence of a smaller relative benefit when counselling is used in addition to pharmacotherapy, and of more intensive counselling compared to a brief counselling intervention. • Individual counselling versus non-active controls: RR 1.57 (95% CI 1.40 to 1.77) • Individual counselling + pharmacotherapy versus non-active controls: RR 1.24 (95% CI 1.01 to 1.51) • Individual counselling versus non-active controls: RR 1.29 (95% CI 1.09 to 1.53)
Stead et al. (2017)	66	Adult smokers.	Smokers met for scheduled meetings and received some form of behavioural intervention (i.e., information, advice, encouragement or cognitive behavioural therapy) over at least two sessions.	No intervention, non-active interventions (i.e. brief advice or self-help), individual counselling sessions.	Abstinence from cigarettes at follow-up at least six months after the start of treatment.	There is reasonable evidence that group therapy is better than non-active controls in helping people stop smoking, although it may be no better than advice from a healthcare provider. There is not enough evidence to determine how effective they are compared to intensive individual counselling. • Group therapy versus no intervention: RR 2.60 (95% CI 1.80 to 3.76) • Group therapy versus self-help programmes: RR 1.57 (95% CI 1.40 to 1.77) • Group therapy versus brief support: RR 1.25 (95% CI 1.40 to 1.77) • Group therapy versus face-to-face individual intervention: RR 0.99 (95% CI 0.76 to 1.28)
Matkin et al. (2019)	104	Current smokers.	Proactive or reactive telephone counselling to assist smoking cessation. The number, duration and content of the telephone calls varied.	Any intervention without telephone counselling (i.e. self-help materials, standard advice or different counselling interventions).	Long-term smoking cessation (i.e. at least six months after the start of intervention).	There is moderate-certainty evidence that proactive telephone counselling aids smokers who seek help from quitlines and increases quit rates in smokers in other settings. There is insufficient evidence that a higher number of calls would result in a more significant effect, although limited evidence suggests interventions offering additional calls may be more effective than those offering one call only. Due to limited studies, evidence was inconclusive on the effect of reactive telephone counselling. • Additional proactive calls for smokers calling quitlines: RR 1.38 (95% CI 1.19 to 1.61) • Proactive calls for smokers not calling quitlines: RR 1.25 (95% CI 1.15 to 1.35)
Tzelepis et al. (2019)	2	Current tobacco smokers.	Real-time video counselling via telemedicine video conferencing technology or other platforms (i.e., Skype, FaceTime, Facebook Messenger, etc.) or alternative forms of video communication.	Telephone counselling for smoking cessation.	Primary outcome: Smoking cessation (e.g. point prevalence, continuous or prolonged abstinence) measured at least six months from baseline.	There is no evidence of a difference in smoking cessation between video counselling and telephone counselling. There is insufficient evidence to draw conclusions regarding the effectiveness of integrating real-time video counselling into the routine practices of quitlines and other smoking cessation services. • Real-time video counselling versus telephone counselling: RR 2.15 (95% CI 0.38 to 12.04)
Whittaker et al. (2019)	26	Current smokers.	Mobile-based programmes (i.e. text messaging, smartphone apps) for smoking cessation.	Text messaging versus minimal smoking cessation support, text messaging in addition to other smoking cessation support, and smartphone app versus less intensive smoking cessation support.	Smoking abstinence at the longest follow-up, and at least six months from baseline.	There is moderate-certainty evidence that text message improves smoking cessation rates, either delivered on their own or as an add-on to other treatments. There is no evidence comparing different intensity text messages on long-term abstinence. There is insufficient evidence to evaluate the effect of mobile app interventions and low-certainty evidence comparing smartphone apps with less intensive support. There is no evidence for a benefit of high-intensity smartphone apps when compared with lower-intensity apps or minimal non-app smoking cessation support. • Text message versus minimal cessation support: RR 1.54 (95% CI 1.19 to 2.00) • Text message + other support versus other support alone: RR 1.59 (95% CI 1.09 to 2.33) • Smartphone app versus lower-intensity support: RR 1.00 (95% CI 0.66 to 1.52)
Barroso-Hurtado et al. (2021)	24	Adult daily smokers (aged 18 and over).	Smartphone apps for smoking cessation. (1) general apps for smoking cessation, which do not include face-to-face contact (GSC-Apps) (2) smoking cessation apps combined with face-to-face intervention (FFSC-Apps)	Other interventions (ie. face-to-face treatment, other mobile apps, brief advice, etc.)	Primary outcome: the effect of smartphone apps for smoking cessation on tobacco use, abstinence, and relapse rates.	Most studies showed that mHealth apps are at least as useful as the control conditions (e.g., brief advice). FFSC-Apps could increase the intensity of smoking cessation treatments because combining an app with face-to-face contact offers more tools to quit. As for GSC-Apps, these kinds of apps can reach more people, increasing the number of people who have access to smoking cessation treatments because they are offered anywhere and at any time. Both kinds of apps could play an important role in the smoking cessation field.
Taylor et al. (2017)	67	Current smokers.	Internet interventions in all settings and from all types of providers.	Non-active controls (i.e. self-help or usual care), active control arm (e.g. telephone or face-to-face counselling), evaluated the addition of an Internet programme plus behavioural support and compared one Internet intervention to another.	Smoking cessation at least six months after the start of the intervention, and longer.	Interactive, tailored Internet-based interventions with or without additional behavioural support are moderately more effective than non-active controls (i.e. printed self-help, usual care) at six months or longer, but there was no evidence that these interventions were better than other active controls (i.e. phone or face-to-face counselling). Non-tailored and interactive Internet interventions appeared no better than non-active controls. Treatment effectiveness in younger people is unknown. • Interactive, tailored Internet intervention versus non-active controls: RR 1.15 (95% CI 1.01 to 1.30) • Internet + behavioural support versus non-active controls: RR 1.69 (95% CI 1.30 to 2.18) • Text message versus minimal cessation support: RR 1.54 (95% CI 1.19 to 2.00)

Do et al. (2018)	108	Adult smokers, followed by youth and other special subpopulations.	Web-based programs, mobile phone-based intervention, computer-assisted intervention, mobile health (mHealth) platforms, and others.	No intervention, usual practice, non-active control, other smoking cessation methods, other eHealth control groups, and interactive tailored web-based compared to an active control group.	Levels of smoking abstinence.	Web-based and mHealth apps may moderately increase smoking cessation rates over short-term periods. Compared to active control groups or other eHealth modes, there is no evidence of the effectiveness of internet-based cessation programs. Tailored web-based and text messaging supports may increase cessation while computer-assisted interventions alone have little impact on smoking abstinence. • Web-based intervention versus non-active controls: RR 2.03 (95% CI 1.70 to 2.03) • mHealth platform versus non-active controls: RR 1.71 (95% CI 1.35 to 2.16) • Computer-assisted interventions versus non-active controls: RR 1.16 (95% CI 1.06 to 1.26)
Livingstone-Banks et al. (2019)	75	Any smokers.	Written materials (i.e. booklets and leaflets), audio or video.	No intervention, tailored self-help programmes versus non-tailored, provision of additional materials to other interventions.	Sustained abstinence, or point prevalence, with a minimum follow-up of six months.	There is moderate-certainty evidence that written self-help materials help more people to stop smoking than no intervention. There is moderate-certainty evidence showing that tailored self-help materials are more effective than no intervention. There is no evidence of benefit comparing tailored self-help materials with untailored materials delivered. • Non-tailored self-help materials versus no materials: RR 1.19 (95% CI 1.03 to 1.37) • Tailored self-help materials versus no materials: RR 1.34 (95% CI 1.19 to 1.51)
Lindson et al. (2019)	37	Tobacco smokers from the general and special populations.	Interventions labelled as either motivational interviewing (MI) (e.g. evoking motivation and confidence to quit, eliciting 'change talk' and supporting self-efficacy) or Motivation Enhancement Therapy (MET), for tobacco smoking cessation.	No intervention, another smoking cessation intervention, lower intensity of MI, and comparison of MI in addition to another smoking cessation treatment versus that smoking cessation treatment alone.	Primary outcome: Smoking cessation at the longest follow-up.	There is insufficient evidence to assess whether MI increases the cessation rate compared with no intervention. MI may modestly increase the likelihood of long-term smoking cessation when used in addition to other interventions or when compared with non-MI cessation interventions. Higher-intensity MI may improve smoking cessation rates compared to lower-intensity MI. • Motivational interviewing versus no intervention: RR 0.84 (95% CI 0.63 to 1.12) • Motivational interviewing versus other intervention: RR 1.24 (95% CI 0.91 to 1.69) • MI + other intervention versus other intervention only: RR 1.24 (95% CI 0.91 to 1.69) • Higher-intensity MI versus lower-intensity MI: RR 1.23 (95% CI 1.11 to 1.37)
Notley et al. (2019)	43	Adult smokers.	Incentive schemes to reward participants for validated cessation and abstinence in smoking cessation programmes.	Any intervention without incentives.	Long-term smoking cessation is assessed at least six months from the start of the intervention.	There is high-certainty evidence that incentives boost long-term cessation rates (≥ 6 months) compared to no incentives. This effect appears to persist following their discontinuation, suggesting that even a short incentive intervention may have long-term benefits. There is moderate-certainty evidence that incentives also boost the long-term cessation rates of pregnant women who smoke, which continues post-partum. • Incentives versus no incentives: RR 1.49 (95% CI 1.28 to 1.73) • Incentives versus no incentives (in pregnant women): RR 2.38 (95% CI 1.54 to 3.69)
Fanshawe et al. (2019)	20	Current adult smokers.	Contests, competitions, lotteries, including Quit & Win contests, to reward cessation or continuous abstinence.	No intervention or non-competition based smoking cessation intervention.	Primary outcome: smoking cessation rate at longest follow-up at least six months from the start of the intervention.	At present, it is impossible to draw any firm conclusions about the effectiveness, or a lack of it, of smoking cessation competitions. This is due to a lack of well-designed comparative studies. Smoking cessation competitions have not been shown to enhance long-term cessation rates. It is also unclear whether the value or frequency of possible cash reward schedules influences the success of competitions.
Jackson et al. (2022)	21	Current tobacco smokers of any age.	Interventions include mindfulness training, acceptance and commitment therapy (ACT), distress tolerance training, or yoga.	No intervention, other smoking cessation intervention, other type of mindfulness intervention (e.g. mindfulness of lower intensity).	Smoking cessation at least six months from the start of the intervention.	There is no clear, long-term benefit of mindfulness-based smoking cessation interventions for increasing smoking quit rates or changing mental health and well-being. However, the evidence was of low and very low certainty due to risk of bias, inconsistency, and imprecision, meaning future evidence may very likely change the interpretation of the results.
Barnes et al. (2019)	14	Smokers who wish to stop smoking.	Hypnotherapy for smoking cessation.	Other approaches to help people stop smoking (i.e. brief advice, or more intensive stop-smoking counselling), or no treatment.	Abstinence from smoking assessed at least six months from the start of treatment.	There is insufficient evidence to support the use of hypnotherapy as a specific treatment for smoking cessation. This review does not demonstrate evidence of a greater long-term benefit of hypnotherapy when compared to other interventions, or to no intervention, for smoking cessation. Most studies did not detect significant differences in quit rates at six months or longer.
Westerdahl et al. (2019)	7	Adult (>18 years) smokers who participate in smoking cessation, respiratory disease screening, or health monitoring programmes.	All interventions in which spirometry results are used to increase motivation to quit smoking.	No intervention or interventions other than those incorporating spirometry results.	Percentage smoking cessation, measured short-term (1 to 6 months) and long-term (more than 6 months).	There is currently only limited evidence to support the use of feedback from spirometry results in addition to smoking cessation counselling with the aim of increasing smoking quit rates. The best way to provide this feedback to the smoker and who should provide the information remains unclear.
Ussher et al. (2019)	24	Tobacco smokers wishing to quit, or recent quitters.	Exercise programme alone or as an adjunct to a smoking cessation intervention.	Non-exercise smoking cessation programme.	Smoking cessation measured after at least six months, using the most rigorous definition available, on an intention-to-treat basis.	There is insufficient evidence to support exercise as a specific aid to smoking cessation. There is no evidence of benefit comparing exercise to other smoking cessation treatments and no evidence of different effects by the type of exercise. Future trials may change these conclusions. • Exercise versus other interventions: RR 1.08 (95% CI 0.96 to 1.22)

Chamberlain et al. (2017)	102	1. Women who are currently smoking or have recently quit smoking and are pregnant, in any care setting. 2. Women who are currently smoking or have recently quit smoking and are seeking a pre-pregnancy consultation.	1. Counselling interventions 2. Health education interventions 3. Feedback interventions 4. Incentive-based interventions 5. Social support (peer, professional and/or partner) 6. Others	Usual care, less intensive interventions, different intervention of the same intensity.	Primary outcome: Smoking abstinence in late pregnancy (point prevalence abstinence): a. self-reported or biochemically validated; b. biochemically validated only	Psychosocial interventions can support women to stop smoking during pregnancy. Evidence from this review suggests that health education and risk advice alone is not sufficient, and any psychosocial support should include additional components, such as counselling, incentives or feedback. The effect of partner support is unclear, and care is needed when including peer- or partner-support components as it may be unhelpful, and may potentially expose vulnerable women to increased risk. • Incentives versus other interventions: RR 2.36 (95% CI 1.36 to 4.09) • Counselling versus usual care: RR 1.44 (95% CI 1.19 to 1.73) • Feedback versus usual care: RR 4.39 (95% CI 1.89 to 10.21)
Spanakis et al. (2022)	12	Adults with severe mental illness and no substance abuse problems (other than nicotine addiction) or learning disability, dementia, other neurocognitive disorders or terminal illness.	Behavioural smoking cessation and reduction interventions (i.e. motivational interviewing, CBT, self-help booklets, advice, etc.).	Any type of behavioural smoking cessation and reduction strategies to each other, usual care or no intervention.	Primary outcome: biochemically verified smoking cessation.	Tailored face-to-face smoking cessation interventions for adults with severe mental ill health appear to be effective when compared with usual care across all time points, but the evidence is equivocal when compared with other active interventions. There is limited evidence comparing tailored online interventions with non-tailored online interventions, and the authors found no studies comparing them with usual care. • Tailored face-to-face intervention versus usual care: RR 2.29 (95% CI 1.38 to 3.81) [medium-term] • Tailored face-to-face intervention versus usual care: RR 1.58 (95% CI 1.09 to 2.30) [long-term] • Tailored online interventions versus non-tailored online interventions: RR 0.87 (95% CI 0.17 to 4.46)
Hawes et al. (2021)	18	Cigarette smokers with schizophrenia or bipolar disorder, or severe mental illness (SMI).	Psychosocial smoking cessation intervention.	Usual care, any type of psychosocial intervention to each other.	Smoking abstinence, smoking reduction, nicotine dependence & quit attempts; with follow-up lengths of less than 6-month, 6-to-11 months, 12 months or longer.	The most promising psychosocial smoking cessation interventions seemed to be initiated in inpatient psychiatric units and employed either a transtheoretical approach (i.e., stage- tailored, decisional balance) or individually-tailored telephone behavioral smoking counseling using a motivational-interviewing framework.
Thurgood et al. (2016)	17	Adult (>18 years) smokers who had recently completed or were currently receiving treatment for a substance use disorder (drugs or alcohol).	Smoking cessation interventions - pharmacological and nonpharmacological approaches.	Usual care.	Primary outcome: biochemically verified (carbon monoxide) self-reported continuous abstinence from smoking, at the 6- or 12-month follow-up.	Smoking cessation interventions using NRT and behavioural support combination appear to increase smoking abstinence in those treated for substance use disorders and have no effect on other substance use treatment outcomes. Continuous abstinence differed significantly between groups at 6 and 12 months, with both intervention groups achieving higher rates than the usual care group. Some studies did not find treatment effective at 6 or 12 months and observed significant effects at shorter-term follow-ups.
Pool et al. (2016)	14	Smokers over 18 years who were HIV-positive.	Interventions include both behavioural and pharmacological elements.	Less intensive control, typically comprising a brief behavioural intervention plus pharmacotherapy.	Primary outcome: tobacco abstinence at least six months after the start of the intervention.	Intense combined pharmacotherapy and behavioural support interventions were effective in achieving abstinence in the short-term (4 weeks to less than 6 months) compared to a control group (i.e. a single brief intervention and pharmacotherapy). However, this effect was not observed for long-term abstinence (> 6 months). The authors could not assess whether interventions combining pharmacotherapy and behavioural support were more effective than either type of support alone. The effects of tailoring, number of contacts and total duration of contact of behavioural support remain unclear.
van Eerd et al. (2016)	16	Smokers with a diagnosis of COPD.	Any pharmacological or/and behavioural treatment as an aid to smoking cessation in participants with COPD.	No treatment or usual care, or one form of behavioural treatment versus a different form of behavioural treatment.	Primary outcome: Percentage of participants with continuous or prolonged abstinence over a period of six months or longer.	Evidence showed higher abstinence rates for high-intensity behavioural treatment over usual care or low-intensity. There is high-quality evidence that a combination of behavioural and pharmacotherapy is effective in helping smokers with COPD quit. Furthermore, the authors conclude that there is no convincing evidence for preferring any particular form of behavioural or pharmacological treatment. • Behavioural intervention versus no intervention or usual care: RR 25.38 (95% CI 8.03 to 80.22)
Smith et al. (2019)	11	Smokers from socioeconomically deprived groups, defined through either individual (eg, educational level, income) or area level indicators (eg, postcode).	A range of interventions including pharmacological and behavioural interventions.	All study types with a pre-intervention/ post-intervention and/or a control group.	Primary outcome: smoking abstinence.	Tailored counselling delivered in a community setting demonstrates the benefits of smoking cessation. The optimal mode and duration of intervention were unclear with findings suggesting varying success for both group and individual behavioural support. The current review demonstrates that certain aspects of behavioural interventions (i.e. incentives, peer facilitators, more intensive counselling) are promising for encouraging cessation in older, deprived smokers.

Table 2. Quality assessment summary.

Article	Items																Quality rating
	1	2*	3	4*	5	6	7*	8	9*	10	11*	12	13*	14	15*	16	
Jackson et al. (2022)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Notley et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Fanshawe et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Matkin et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Tzelepis et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Lindson et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Livingstone-Banks et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Whittaker et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Ussher et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Barnes et al. (2019)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Taylor et al. (2017)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Chamberlain et al. (2017)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Pool et al. (2016)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
van Eerd et al. (2016)	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	High
Stead et al. (2017)	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	Y	Moderate
Do et al. (2018)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	PY	Moderate
Lancaster & Stead (2017)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	N	Y	Low
Spanakis et al. (2022)	Y	Y	Y	Y	Y	Y	N	Y	Y	N	Y	Y	Y	Y	Y	Y	Low
Thurgood et al. (2016)	Y	N	Y	Y	Y	Y	Y	Y	Y	N	NC	NC	Y	Y	Y	Y	Low
Barroso-Hurtado et al. (2021)	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	NC	NC	Y	N	N	Y	Low
Westerdahl et al. (2019)	Y	N	Y	Y	Y	Y	N	Y	Y	N	NC	NC	Y	N	N	Y	Critically low
Hawes et al. (2021)	Y	Y	Y	Y	Y	Y	N	Y	Y	N	NC	NC	N	Y	Y	Y	Critically low
Smith et al. (2019)	Y	Y	N	Y	N	N	N	Y	Y	N	NC	NC	N	Y	N	Y	Critically low

Indicator - N: No, Y: Yes, PY: Probably Yes, NC: Not conducted

* Critical items that have critical effect on the overall quality of systematic reviews.

Item 1. Did the research questions and inclusion criteria for the review include the components of PICO?

Item 2. Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?

Item 3. Did the review authors explain their selection of the study designs for inclusion in the review?

Item 4. Did the review authors use a comprehensive literature search strategy?

Item 5. Did the review authors perform study selection in duplicate?

Item 6. Did the review authors perform data extraction in duplicate?

Item 7. Did the review authors provide a list of excluded studies and justify the exclusions?

Item 8. Did the review authors describe the included studies in adequate detail?

Item 9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?

Item 10. Did the review authors report on the sources of funding for the studies included in the review?

Item 11. If meta-analysis was performed, did the review authors use appropriate methods for statistical combination of results?

Item 12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?

Item 13. Did the review authors account for RoB in primary studies when interpreting/discussing the results of the review?

Item 14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?

Item 15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?

Item 16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?

As for telephone counselling or quitlines, there are two types of quitlines which are the 'proactive quitlines' and 'reactive quitlines'. In proactive quitlines, healthcare providers call smokers on a pre-arranged basis; while in reactive quitlines, smokers contact the helpline to ask for assistance or support. Proactive quitlines have received more evaluation compared to reactive quitlines as they are more easily controlled (MOH Malaysia, 2016). Matkin et al. (2019) conclude that proactive quitlines help increase quit rates in smokers in the long term. Smokers who were provided with more than one call may have more chance of quitting than those with only a single session. Currently, there is limited evidence of the effectiveness of reactive quitlines hence conclusion cannot be made. Providing additional proactive calls in reactive quitlines may help increase smoking cessation rates in which the RR was 1.38 (95% CI 1.19 to 1.61). People who have not contacted quitlines but receive other cessation interventions also can be given proactive telephone counselling, and these individuals may or may not be motivated to try to quit. The RR for providing proactive telephone counselling for smokers not contacted quitlines was 1.25 (95% CI 1.15 to 1.35).

The counselling approaches go in line with the CPG in which individual, group and telephone counselling are effective and should be used in smoking cessation interventions. On the other hand, Tzelepis et al. (2019) assessed the effectiveness of real-time video counselling for smoking cessation. There is very little evidence on the effectiveness of real-time video counselling compared to telephone counselling in which the RR was 2.15 (95% CI 0.38 to 12.04). Since the systematic review found only two RCTs and both studies use the same comparator, there is insufficient evidence from which to draw valid conclusions about the effectiveness of incorporating real-time video counselling into the standard practices of other smoking cessation services.

According to Whittaker et al. (2019), there is moderate-certainty evidence that text messages, with or without additional intervention improve long-term abstinence rates. The RR for text message alone versus minimal support was 1.54 (95% CI 1.19 to 2.00) and the RR for text message in addition to other smoking cessation interventions versus other interventions alone was 1.59 (95% CI 1.09 to 2.33). There is no evidence that different intensities of text messages affect the long-term cessation rate. There is insufficient evidence to evaluate the effect of mobile app-based intervention for smoking cessation. The RR for smartphone app versus lower-intensity smoking cessation intervention was 1.00 (95% CI 0.66 to 1.52) but the evidence was of very low certainty.

Barroso-Hurtado et al. (2021) also reviewed the evidence on the effectiveness of smoking cessation apps and found that these apps produced abstinence rates ranging from 36% to 100% at the end of treatment, making them at least as effective as the control intervention (i.e., brief advice). The study classified available app-based intervention as general smoking cessation apps (GSC-Apps) which has no face-to-face contact with healthcare professional, and smoking cessation app combined with face-to-face contact (FFSC-Apps). The authors find both kinds of mHealth apps are promising tools as they may complement established conventional cessation therapy, although FFSC-Apps could provide more intensity. However, only a few studies in this review included a 6-month or longer follow-up hence the evaluation of long-term abstinence from the treatment is limited.

Taylor et al. (2017) noted that non-interactive, non-tailored Internet interventions were no better than non-active controls. There is moderate-certainty evidence that interactive, tailored Internet-based interventions, either delivered on their own or as an adjunct to other interventions, are more effective than non-active controls at six months or longer. The RR for Internet intervention alone versus non-active controls was 1.15 (95% CI 1.01 to 1.30) and the RR for Internet intervention added to other behavioural support versus non-active control was 1.69 (95% CI 1.30 to 2.18). Interactive, tailored Internet-based interventions appear slightly better than non-interactive, non-tailored Internet interventions. However, tailored Internet-based interventions appeared no better than active controls (i.e., counselling) in which the RR for this comparison was 0.92 (95% CI 0.78 to 1.09). The pieces of evidence found are mostly on adults while effects on younger people are unknown or limited.

Electronic-based (eHealth) interventions for smoking cessation were assessed by Do et al. (2018) which include web-based, phone-based, mobile-based (mHealth) and computer-assisted interventions. The findings support that interactive and tailored web-based, mHealth platforms and text messaging may increase the smoking cessation rate. Evidence shows the approaches led to six-month or longer quit rates compared to non-active control. The RR for web-based intervention versus non-active controls was 2.03 (95% CI 1.70 to 2.03). The RR for the mHealth platform versus non-active control was 1.71 (95% CI 1.35 to 2.16). Computer-assisted interventions alone do not have much of an effect on smoking abstinence compared to non-active control in which the RR was 1.16 (95% CI 1.06 to 1.26). Little to no benefit was found regarding the effectiveness of internet-based interventions when compared to active control or other eHealth interventions.

To summarise online smoking cessation interventions such as text messages, web-based, internet-based, and mobile-based are effective in assisting smokers to quit smoking, which aligns with the 2016 CPG. As for app-based interventions, there is currently limited evidence of their effectiveness for long-term abstinence but is considered a promising tool in the future. Interactive and tailored Internet interventions for smoking cessation appear to have better effects than non-interactive and non-tailored ones.

Self-help materials are commonly provided to all smokers seeking help to quit smoking. Livingstone-Banks et al. (2019) evaluated that printed self-help materials help people to quit smoking compared to no intervention in the medium to long term. The RR for non-tailored materials versus no materials was 1.19 (95% CI 1.03 to 1.37), meanwhile, the RR for tailored materials versus no materials was 1.34 (95% CI 1.19 to 1.51). However, there is no evidence that self-help materials provide additional benefits to other smoking cessation interventions.

Aside from self-help materials, MI techniques also have already been incorporated into the CPG as one of the standardised behavioural approaches (MOH Malaysia, 2016). Lindson et al. (2019) reported that there is not enough evidence to evaluate whether motivational interviewing (MI) increases the smoking cessation rate compared to no intervention, however, this was of low-certainty evidence. The RR for MI versus no intervention was 0.84 (95% CI 0.63 to 1.12). When compared to other interventions or used in conjunction with them, MI may modestly increase the potential for long-term smoking cessation. The RR for MI

versus other intervention was 1.24 (95% CI 0.91 to 1.69) while the RR for MI added to other intervention versus other intervention only was 1.07 (95% CI 0.85 to 1.36). Evidence is also in favour of more intensive MI compared to the less intensive ones in which the RR was 1.23 (95% CI 1.11 to 1.37).

As per Notley et al. (2019), there is high-certainty evidence that incentives may improve long-term smoking cessation rates compared to no incentives. The RR for this comparison was 1.49 (95% CI 1.28 to 1.73). The author focuses mainly on the financial incentives (i.e. money, vouchers). There were concerns regarding the financial implications on the provider in which the author discussed the reported costs from his gathered pieces of evidence. It was found that the cost per quitter from the incentive intervention is lower compared to quitting with other interventions (e.g. nicotine gum, varenicline, free cessation aids). One even noted that in Thailand, the intervention complies with the WHO's ranking of "very cost-effective". Still, the affordability of this intervention may vary across different countries and should be studied in the future. There were also concerns that the long-term smoking cessation effect might not last long once the rewards were discontinued. However, incentives can support the initiation cessation within individuals, and they may adapt to this change over time. This also moderately applies to pregnant smokers in which the effect continues post-partum (10 to 24 weeks). The RR for incentives versus no incentives in smoking pregnant women was 2.38 (95% CI 1.54 to 3.69). This suggests that incentives have a significant impact on sustained smoking cessation and long-term effects can be maintained. The authors however cannot conclusively link the incentives' value or frequency to the efficiency of the intervention.

On the other hand, smoking cessation competitions such as Quit & Win contests, have not been shown to enhance long-term cessation rates. As for the incentive, it is unclear whether the value or frequency of the reward affects the outcome measure (Fanshawe et al., 2019). As for mindfulness-based interventions, clear evidence of the long-term effect of the intervention on increasing the smoking cessation rate also were not found. The evidence was of low and very low certainty due to the risk of bias, imprecision and inconsistency (Jackson et al., 2022). Same as Barnes et al. (2019), the authors found no evidence of a specific effect in providing hypnotherapy for long-term smoking cessation. The authors reported that if hypnotherapy was able to increase the smoking quit rate compared to no intervention or brief advice, it may be because of nonspecific factors such as prolonged contact with a therapist.

Westerdahl et al. (2019) reviewed the benefit of including feedback on spirometry results (FEV1 and/or lung age) in smoking cessation counselling. A small proportion of the evidence shows that it demonstrated a benefit, suggesting the potential for its inclusion as a future intervention. The effect of incorporating spirometry results in counselling should be tested more in real-life settings and explored in future studies to enhance the quality of evidence.

Another intervention that has limited evidence of its effectiveness for smoking cessation is exercise interventions. The RR for exercise intervention alone or in conjunction with other interventions versus other interventions alone was 1.08 (95% CI 0.96 to 1.22. It was demonstrated that acute bouts of exercise may be beneficial in reducing craving and withdrawal symptoms. The author remarked that if exercise plays a role in helping individuals quit smoking, a continuous commitment is likely essential for

sustaining smoking cessation (Ussher et al., 2019). Exercise does not appear to be superior to other smoking cessation therapies, however, its potential should not be completely dismissed as an adjunct intervention alongside other treatments.

Findings from most studies suggest that behavioural interventions of any type or component are effective in increasing long-term smoking cessation rates compared to no intervention. However, it is difficult to determine which intervention or components are the most effective. As discussed by Stead et al. (2017), a few problems should be noted in conducting a systematic review of behavioural interventions. One of them is the choice of an appropriate control condition for behavioural intervention, which may cause difficulties in evaluating the efficacy of the intervention. However active interventions such as counselling and online interventions appear to be more effective than non-active interventions such as printed self-help materials and brief advice. For better outcomes, interactive, tailored and high-intensity interventions using a single or a combination of treatments may be required.

The next part of the discussion will focus on findings from studies that discuss the interventions for special populations as extra care should be given when treating certain special populations.

One of the special populations focused on is pregnant women and some psychosocial interventions recommended from the 2019 CPG were advice, self-help materials, and counselling sessions. Evidence from Chamberlain et al., (2017) reveals that providing advice regarding health-related risks or health education alone is not enough. Any psychosocial assistance should include elements like incentives, counselling, or feedback. There is high-certainty evidence that incentives had a large effect and the RR for incentives compared to alternative interventions was 2.36 (95% CI 1.36 to 4.09). Counselling or providing feedback (i.e., fetal health, carbon monoxide) works best when tailored or combined with other approaches. Compared to usual care, the RR for counselling was 1.44 (95% CI 1.19 to 1.73) while the RR for feedback was 4.39 (95% CI 1.89 to 10.21). The effect of social support (partner, peer, healthcare provider) on smoking cessation in pregnant women is unclear.

Spanakis et al. (2022) and Hawes et al. (2021) analysed behavioural smoking cessation interventions for smokers with serious mental illness (SMI). It is important that all psychiatric patients who smoke be asked to quit when seen in psychiatric services (MOH Malaysia, 2016). Hawes et al. (2021) also acknowledged this by pointing out that the most promising behavioural interventions for smoking cessation appear to be the ones that were initiated in inpatient psychiatric facilities. Findings from this review showed that there is insufficient evidence to support any particular durations, intensities or modes of psychosocial interventions for smokers with SMI (Hawes et al., 2021).

However, tailored, face-to-face intervention for adult smokers with SMI is effective when compared with usual care but appears to be indefinite when compared with other active interventions. The RR value for tailored face-to-face intervention versus usual care was 2.29 (95% CI 1.38 to 3.81) in the medium term and 1.58 (95% CI 1.09 to 2.30) in the long term. There is no evidence of benefit found comparing tailored online interventions with non-tailored online interventions, in which the RR was 0.87 (95% CI 0.17 to 4.46) in the medium term (Spanakis et al., 2022).

Thurgood et al. (2016) noted that pharmacotherapy and behavioural support combinations were beneficial in adult smokers receiving treatment for substance use disorder (SUD). Combination smoking cessation intervention increases the abstinence rate and does not affect other substance use treatment outcomes. Smokers receiving behavioural support for 6-months and 12-months have significant differences in continuous abstinence, but both groups achieve higher abstinence rates than the usual care. Evidence that did not find treatment effective in the long-term also observed significant effects at follow-ups of less than 6-months. This demonstrates that behavioural interventions can increase the abstinence rate among smokers with SUD.

Pool et al. (2016) assessed smoking cessation interventions in people living with HIV and AIDS (PLWHA). Compared to the control group which typically consisted of a single brief intervention and pharmacotherapy, this review found that more intensive, combined behavioural and pharmacological interventions were effective in increasing the chance of achieving abstinence in the short-term (4 weeks to 6 months). However, this effect was not observed for long-term abstinence (greater than six months). The authors were unable to assess whether combined pharmacotherapy and behavioural interventions were more effective than either type of support alone. They also did not discuss any specific intervention that is best for PLWHA. It is unclear on the effects of tailoring, total amount and duration of contact of behavioural support.

Van Eerd et al. (2016) found high-quality evidence that smokers with chronic obstructive pulmonary disease (COPD) can successfully quit when provided a combination of pharmacotherapy and behavioural treatment. The RR for behavioural intervention versus no treatment or usual care was 25.38 (95% CI 8.03 to 80.22). Behavioural interventions of high-intensity increased abstinence rates compared to usual care and low-intensity behavioural intervention. Evidence that compared one high-intensity behavioural treatment with another did not provide sufficient data to draw clear conclusions. The authors also conclude that there is little support for favouring any specific pharmacological or behavioural treatment.

According to Smith et al. (2019), underprivileged older smokers may benefit from tailored counselling delivered in the community setting. The author defines underprivileged older smokers as smokers aged 40 years and above who are socioeconomically deprived (i.e. their income or educational level). Findings reveal that both group and one-to-one behavioural support have varied degrees of success, making it unclear what the best duration and components of intervention should be. The study demonstrates that several behavioural intervention components such as incentives, peer facilitators and intensive counselling, are promising for helping older, underprivileged smokers quit. There is a clear lack of evidence from large-scale trials on effectiveness in a lung screening context for this population. The review highlights the lack of solid evidence for behavioural smoking cessation interventions that are effective for the lung screening eligible population of older, deprived smokers.

From the evidence, smokers in special populations have more chance to quit smoking when provided behavioural interventions than pharmacotherapy alone. Same as in the general population, it is not sure which intervention is best for them, but tailored or higher-intensity interventions appear to have better effects than non-tailored or lower-intensity ones.

Conclusion

Counselling sessions, online interventions, self-help materials and MI may increase cessation rates in the long term, if not, short term. It is unclear which type of intervention is best for the general and special populations. However, counselling sessions of any form do demonstrate the strongest evidence of benefit in smokers trying to quit; may it be individual, group, face-to-face or online counselling. Some findings on interventions that can be updated in the CPG include app-based interventions, incentives, providing feedback on spirometry results, and exercise. As for behavioural interventions for special populations, updates may include people living with HIV and AIDS, smokers with COPD, and underprivileged older smokers.

Authors Contributions

Conceptualization, N.S. and M.H.; methodology, N.S. and A.N.; writing, N.S.; supervision, M.H. and S.Z. All authors have read and agreed to the updated version of the manuscript.

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Conflict of Interest

None declared.

References

Barnes, J., McRobbie, H., Dong, C. Y., Walker, N., & Hartmann-Boyce, J. (2019). Hypnotherapy for smoking cessation. *The Cochrane Database of Systematic Reviews*, *6*(6), CD001008. https://doi.org/10.1002/14651858.CD001008.pub3

Barroso-Hurtado, M., Suárez-Castro, D., Martínez-Vispo, C., Becoña, E., & López-Durán, A. (2021). Smoking Cessation Apps: A Systematic Review of Format, Outcomes, and Features. *International Journal of Environmental Research and Public Health*, *18*(21). https://doi.org/10.3390/ijerph182111664

CDC. (2020). *Health effects - smoking and tobacco use*. Centers for Disease Control and Prevention. https://www.cdc.gov/tobacco/basic_information/health_effects/index.htm

Chamberlain, C., Porter, J., Coleman, T., Sm, P., Thomas, J., Je, M., Chamberlain, C., Porter, J., Coleman, T., Sm, P., Thomas, J., & Je, M. (2017). *Psychosocial interventions for supporting women to stop smoking in pregnancy (Review)*. https://doi.org/10.1002/14651858.CD001055.pub5.www.cochranelibrary.com

Cheung, K. L., De Ruijter, D., Hiligsmann, M., Elfeddali, I., Hoving, C., Evers, S. M. A. A., & De Vries, H. (2017). Exploring consensus on how to measure smoking cessation. A Delphi study. *BMC Public Health*, *17*(1). https://doi.org/10.1186/S12889-017-4902-7

Cutler, D. M. (2004). Behavioral Health Interventions: What Works and Why. *Critical Perspectives on Racial and Ethnic Differences in Health in Late Life*, *643*, *674*.

https://www.researchgate.net/publication/228378967

Do, H. P., Tran, B. X., Le Pham, Q., Nguyen, L. H., Tran, T. T., Latkin, C. A., Dunne, M. P., & Baker, P. R. (2018). Which eHealth interventions are most effective for smoking cessation? A systematic review. *Patient Preference and Adherence*, *12*, 2065–2084. https://doi.org/10.2147/PPA.S169397

Fanshawe, T. R., Hartmann-Boyce, J., Perera, R., & Lindson, N. (2019). Competitions for smoking cessation. *Cochrane Database of Systematic Reviews*, *2019*(2). https://doi.org/10.1002/14651858.CD013272

Hartmann-Boyce, J., Lancaster, T., & Stead, L. F. (2014). Print-based self-help interventions for smoking cessation. *The Cochrane Database of Systematic Reviews*, *2017*(6). https://doi.org/10.1002/14651858.CD001118.PUB3

Hawes, M. R., Roth, K. B., & Cabassa, L. J. (2021). Systematic Review of Psychosocial Smoking Cessation Interventions for People with Serious Mental Illness. *Journal of Dual Diagnosis*, *17*(3), 216. https://doi.org/10.1080/15504263.2021.1944712

Jackson, S., Brown, J., Norris, E., Livingstone-Banks, J., Hayes, E., & Lindson, N. (2022). Mindfulness for smoking cessation. *The Cochrane Database of Systematic Reviews*, *4*(4), CD013696. https://doi.org/10.1002/14651858.CD013696.pub2

Lancaster, T., & Stead, L. F. (2017). Individual behavioural counselling for smoking cessation. *Cochrane Database of Systematic Reviews*, *2017*(3). https://doi.org/10.1002/14651858.CD001292.PUB3

Lindson, N., Thompson, T. P., Ferrey, A., Lambert, J. D., & Aveyard, P. (2019). Motivational interviewing for smoking cessation. *Cochrane Database of Systematic Reviews*, *2019*(7). https://doi.org/10.1002/14651858.CD006936.pub4

Matkin, W., Ordóñez-Mena, J. M., & Hartmann-Boyce, J. (2019). Telephone counselling for smoking cessation. *Cochrane Database of Systematic Reviews*, *2019*(5). https://doi.org/10.1002/14651858.CD002850.pub4

Ministry of Health (MOH) Malaysia. (2016). *CLINICAL PRACTICE GUIDELINES ON TREATMENT OF TOBACCO USE DISORDER*. http://www.moh.gov.my

Notley, C., Gentry, S., Livingstone-Banks, J., Bauld, L., Perera, R., & Hartmann-Boyce, J. (2019). Incentives for smoking cessation. *Cochrane Database of Systematic Reviews*, *2019*(7). https://doi.org/10.1002/14651858.CD004307.pub6

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., … Moher, D. (2021). The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*, *372*. https://doi.org/10.1136/BMJ.N71

Pool, E. R., Dogar, O., Lindsay, R. P., Weatherburn, P., & Siddiqi, K. (2016). Interventions for tobacco use cessation in people living with HIV and AIDS. *The*

Cochrane Database of Systematic Reviews, *2016*(6). https://doi.org/10.1002/14651858.CD011120.PUB2

Smith, P., Poole, R., Mann, M., Nelson, A., Moore, G., & Brain, K. (2019). Systematic review of behavioural smoking cessation interventions for older smokers from deprived backgrounds. *BMJ Open*, *9*(11), e032727. https://doi.org/10.1136/bmjopen-2019-032727

Smith, V., Devane, D., Begley, C. M., & Clarke, M. (2011). Methodology in conducting a systematic review of systematic reviews of healthcare interventions. *BMC Medical Research Methodology*, *11*(1), 1–6. https://doi.org/10.1186/1471-2288-11-15/TABLES/2

Spanakis, P., Peckham, E., Young, B., Heron, P., Bailey, D., & Gilbody, S. (2022). A systematic review of behavioural smoking cessation interventions for people with severe mental ill health-what works? *Addiction (Abingdon, England)*, *117*(6), 1526–1542. https://doi.org/10.1111/add.15724

Stead, L. F., Carroll, A. J., & Lancaster, T. (2017). Group behaviour therapy programmes for smoking cessation. *Cochrane Database of Systematic Reviews*, *2017*(3). https://doi.org/10.1002/14651858.CD001007.pub3

Tawfik, G. M., Dila, K. A. S., Mohamed, M. Y. F., Tam, D. N. H., Kien, N. D., Ahmed, A. M., & Huy, N. T. (2019). A step by step guide for conducting a systematic review and meta-analysis with simulation data. *Tropical Medicine and Health*, *47*(1), 1–9. https://doi.org/10.1186/S41182-019-0165-6/FIGURES/4

Taylor, G. M. J., Dalili, M. N., Semwal, M., Civljak, M., Sheikh, A., & Car, J. (2017). Internet-based interventions for smoking cessation. *The Cochrane Database of Systematic Reviews*, *9*(9). https://doi.org/10.1002/14651858.CD007078.PUB5

Thurgood, S. L., McNeill, A., Clark-Carter, D., & Brose, L. S. (2016). A systematic review of smoking cessation interventions for adults in substance abuse treatment or recovery. *Nicotine and Tobacco Research*, *18*(5), 993–1001. https://doi.org/10.1093/ntr/ntv127

Tzelepis, F., Paul, C. L., Williams, C. M., Gilligan, C., Regan, T., Daly, J., Hodder, R. K., Byrnes, E., Byaruhanga, J., McFadyen, T., & Wiggers, J. (2019). Real-time video counselling for smoking cessation. *Cochrane Database of Systematic Reviews*, *2019*(10). https://doi.org/10.1002/14651858.CD012659.pub2

Ussher, M. H., Faulkner, G. E. J., Angus, K., Hartmann-Boyce, J., & Taylor, A. H. (2019a). Exercise interventions for smoking cessation. *Cochrane Database of Systematic Reviews*, *2019*(10). https://doi.org/10.1002/14651858.CD002295.pub6

van Eerd, E. A. M., van der Meer, R. M., van Schayck, O. C. P., & Kotz, D. (2016). Smoking cessation for people with chronic obstructive pulmonary disease. *Cochrane Database of Systematic Reviews*, *2016*(8). https://doi.org/10.1002/14651858.CD010744.pub2

Westerdahl, E., Engman, K. O., Arne, M., & Larsson, M. (2019a). Spirometry to increase smoking cessation rate: A systematic review. *Tobacco Induced Diseases*, *17*, 31.

https://doi.org/10.18332/tid/106090

Whittaker, R., McRobbie, H., Bullen, C., Rodgers, A., Gu, Y., & Dobson, R. (2019). Mobile phone text messaging and app-based interventions for smoking cessation. *The Cochrane Database of Systematic Reviews*, *10*(10). https://doi.org/10.1002/14651858.CD006611.PUB5

World Health Organization (WHO). (2022). *Tobacco*. https://www.who.int/news-room/fact-sheets/detail/tobacco

Appendix 1. Syntax or keywords for search strategy

#1, Behavioural interventions:

("behavioural intervention*"[Title/Abstract] OR "behavioural therap*"[Title/Abstract] OR "behavioural treatment*"[Title/Abstract] OR "behavioural approach*"[Title/Abstract] OR "behavioural therap*"[Title/Abstract] OR "cognitive behavioural therapy"[Title/Abstract] OR CBT [Title/Abstract] OR "online smoking cessation intervention*"[Title/Abstract] OR "online intervention*"[Title/Abstract] OR "telephone-based intervention*"[Title/Abstract] OR "webbased intervention*"[Title/Abstract] OR "internet-based intervention*"[Title/Abstract] OR "app-based intervention*"[Title/Abstract] "media intervention*"[Title/Abstract] OR "mobile phone"[Title/Abstract] OR "text messaging"[Title/Abstract] OR counselling[Title/Abstract])

#2, Smoking cessation:

("tobacco smoking"[Title/Abstract] OR "cigarette smoking"[Title/Abstract] OR "smoking cessation"[Title/Abstract] OR "tobacco cessation"[Title/Abstract] OR "cigarette cessation"[Title/Abstract] OR "nicotine cessation"[Title/Abstract] OR "smoking abstinence"[Title/Abstract] OR "tobacco abstinence"[Title/Abstract] OR "cigarette abstinence"[Title/Abstract] OR "nicotine abstinence"[Title/Abstract] OR "stop smoking"[Title/Abstract] OR "quit smoking"[Title/Abstract] OR "quitting smoking"[Title/Abstract])

#3, Final search strategy:

#1 AND #2

Filter: Systematic review; English language; date of publication (January 2016 – November 2022)

Journal of Pharmacy

Journal of Pharmacy

Assessment of Methods to Measure Adherence of Antidepressants: A Systematic Review

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Abstract

Introduction: Adherence towards antidepressant agents is a vital element in effectively managing depression. Non-adherence of antidepressants can lead to a recurrence of depressive symptoms and decreased treatment effectiveness. Adherence is assessed using various types of measures. This study aims to evaluate the different methods used to assess the adherence towards antidepressants on adults with depression**. Method:** "This systematic review adhered to the guidelines outlined in the PRISMA statement. "PubMed, Cochrane Library, and Scopus are searching from 2013 to 2023 for articles that studied or reported on antidepressant adherence measures in adults with depression. Two authors conducted independent screenings of the articles against the eligibility criteria, examining titles, abstracts, and full texts. "The risk of bias for all included studies were assessed using the Joanna Briggs Institute (JBI) critical appraisal checklists. "Information from all the selected articles was extracted using a predefined table. **Results:** 15 studies met the eligibility criteria. When measuring adherence towards antidepressant at initiation and/or implementation phase, "self-report methods such as Medication Adherence Rating Scale (MARS) demonstrated acceptable reliability and validity, while Brief Medication Questionnaire (BMQ by Svarstad et al.), Morisky Medication Adherence Questionnaire (MAQ), and Brief Adherence Rating Scale (BARS) showed good validity, and Morisky Medication Adherence Scale (MMAS), Morisky Green Levine Adherence (MGLA), Beliefs about Medicine Questionnaire (BMQ by Horne et al.) and Drug Attitude Inventory (DAI-10) showed good reliability." **Conclusion**: This study found a diverse range of methods to measure adherence towards antidepressant in adults. Self-report assessments, particularly in primary care and psychiatric settings, emerged as the most practical tools followed by clinician-rating scale, pharmacy refill data, adherence scale, pill count, and average serum level. No single measure with consistently shown strong reliability and validity across different adherence stages, highlighting the need for a combined approach.

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Introduction

Depression is a highly widespread mental health illness worldwide, and the utilization of antidepressants is considered a fundamental component in it's -management. Depression is one ofpsychiatric condition marked by prolonged sadness and a reduced motivation in engaging in pleasurable activities (Chand & Arif, 2021). Depressive illnesses are characterized by persistently feelings of sadness, emptiness, or irritabilities, for constant two weeks duration, which associated with physical and mental changes that would substantially influence functional abilities on individuals (Ormel et al., 2019).

Compliance with pharmacological regimeis crucial towards the successful management of major mental illness such as depression. Adherence is defined as the degree to which an individual's behaviours correlate with health-related instructions or suggestions provided by a healthcare professional in relation to a particular disease or disorder condition (Gast & Mathes, 2019). The lack of adherence on following instruction of treatments has been found to be associated with a deterioration in mental well-being and an increased likelihood of experiencing relapse of depressive symptoms (Stewart et al., 2022).

When examining the rates of non-adherence towards antidepressant medication over a six-month period, it is seen that there is a little difference between psychiatric groups (52%) and primary care populations (46.2%). It indicates around 50% of patients showed non-adherence towards antidepressant medication regardless of whether they are from primary or psychiatric care settings (Sansone & Sansone, 2019). On the other hand, the prevalence of non-adherence in older individuals in the United States varies from 29% to 40% "(Sirey et al., 2017).

Research undertaken in both primary care and psychiatric settings has revealed that most patients with diagnosis of major depressive disorder (MDD) show poor compliance to their antidepressant. It shows that a significant proportion of individuals diagnosed with MDD exhibit poor compliance towards antidepressant (Dell'Osso et al., 2020). While the importance of adherence is well-established, the choice of measurement method is a critical factor in understanding and addressing this issue. The "gold standard" for evaluating medication adherence has not been established yet. Hence, choosing between at least two approaches whether direct or indirect methods can produce more accurate outcome. (Jimmy & Jose, 2020).

Multiple measurements with various methods are employed to evaluate adherence towards antidepressant medication. These may encompass self-report questionnaires, electronic monitoring, pharmacy refill data, and clinical assessments (Lam & Fresco, 2015). These measurements offer some assistance in determining how closely patients adhere to their prescription schedule or prescribed medication regime. Different adherence measures may lead to variability in adherence reports, incomparable adherence outcomes, thus, it causes the elevation of an inaccurate conclusion. Therefore, this systematic review aims to evaluate the different methods used to assess the adherence of antidepressants by using different objective and subjective measures.

"The introductory section plays a crucial role in contextualising the study and underscoring its significance. State briefly the purpose, and rationale for the study or observation. Avoid a review of the subject by confining to only relevant information and references. Do not include data or conclusions from the work being cited. Citations are written according to **APA 7th style.** Kindly refer to the example of references in this template. Examples of in-text citations (Almanasef, 2021; Chang *et al*., 2020; Chung *et al*., 2021; Devraj *et al*., 2019; Liu *et al*., 2022; Martí-García *et al*., 2023; Morse, 2000; Zaini *et al*., 2018).

Materials and methods

Methods

Protocol

Articles published from January 2013–November 2023. These databases were searched for articles that studied or reported on antidepressant adherence

measures among people with depression

Search Strategy

This systematic study followed the guidelines and fundamental principles outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2021)."The principal source of literature was from electronic bibliographic databases using a comprehensive search strategy. Three databases; PubMed, Library Cochrane and Scopus were searched for articles.

The search medical subject headings (MeSH) phrases for four key domains were combined: adherence, antidepressant, depression, and methods. Domain one included keywords: adherence, compliance, nonadherence, non-adherence, noncompliance, and non-compliance. Domain two included keywords: antidepressant, antidepressive agents, and antidepressant medicines. Domain three included keywords: depression, major depressive disorders, unipolar depression, and bipolar depression. Domain four included keywords: methods, techniques, procedures, and measures. These four domains were joined together using Boolean operators such as "OR" or/and "AND" to make sure the search strategy used was efficacious.

Eligibility criteria

This systematic review examined the eligibility criteria for the articles. Articles were included based on inclusion criteria such as the aged between 18 years and above. The article evaluated about antidepressant adherence in adults with depression were also included. Study design was not limited to only randomised controlled trial (RCT) however other study designs such as cohort, cross-sectional, and quasi-experimental" studies are also included. Furthermore, only studies that published in English Language were included for better data sources extraction. This review excluded studies with participants whomwith other types of comorbidities, children oradolescents, and pregnant women. If the studies reported about antidepressant adherence measured in depression with comorbidity, the studies were also excluded. Further exclusion criteria include study protocols, conference proceedings, editorials, or letters and non-English Language published articles.

Study selection

The first reviewer systematically searched for articles in accordance with the PRISMA guidelines. At first, the papers were evaluated by examining their titles. If the titles indicated a connection to the study's objectives, abstracts were further examined for more details. Upon confirming that the abstracts satisfied the eligibility requirements, full-text articles were obtained to extract farther information. Duplicate articles were removed using Mendeley. The first reviewer conducted the primary data extraction, while the second reviewer verified and validated the qualifying articles by cross-checking. All discrepancies were addressed and discussed as necessary.

Data extraction

Data extracted from the studies include various key elements, such as authors, year publication, study region, study setting (e.g. psychiatric wards, primary care settings), study design (e.g. whether it was a randomised controlled trial), duration that subjects were followed-up, method to measure medication adherence, "psychometric properties of these measures, outcome of adherence, "group of the subjects, and specific phase of adherence. The systematic review employed the adherence phase framework as outlined. We classified studies into specific adherence phases. The initiation phase addressed participants newly prescribed or initiating antidepressant therapy. The implementation phase scrutinised adherence among those who had initiated antidepressant treatment. The discontinuation phase investigated medication adherence when patients ceased taking antidepressant medications (Vrijens et al., 2012).

Risk of bias in individual studies

The risk of bias for all included studies were assessed using the Joanna Briggs Institute (JBI) critical

appraisal checklists (Joanna Briggs Institute, 2017). Scores of '1' were given if the studies fulfil the stated criteria of the checklist, '0.5' if unclear, and '0' if not. After that, the total score was calculated and converted into percentage. Studies with a percentage <50% were categorised as having a high risk of bias, indicative of a low study quality. For percentages falling within the range of 50% to 70%, a moderate risk of bias was assigned, implying a moderate study quality. Conversely, studies attaining a percentage >70% were designated as possessing a low risk of bias, indicative of a high study quality. Any disagreements will be discussed between the two authors. The results were then visualised as traffic-light plots by using "Risk-of-bias VIsualization (robvis) tool (McGuinness and Higgins, 2020)."

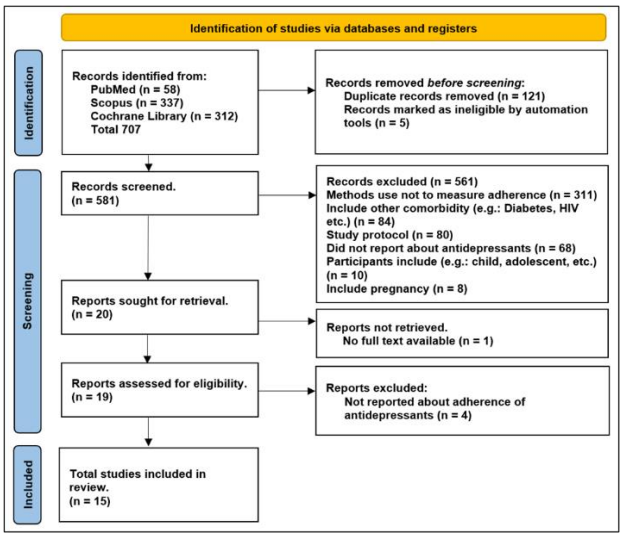

Fig. 1: Flowchart of the study selection process

Outcome of interest

Validity refers to the extent to which a tool accurately measures the intended target of assessment. Meanwhile, reliability pertains to the degree of consistency in findings when an experiment, test, or other measuring technique is performed multiple times (Asunta et al., 2019). Additionally, various psychometric properties, such as correlation or concordance between different adherence measures, were examined in studies. The findings were presented in a structured manner, consisting of a summary of the results and an evaluation of the reliability and validity of the measurements. Following that, the text provided a comprehensive account of the frequency of use for various assessment methods in evaluating adherence to antidepressants in patients with depression.

Results and discussion

Study selection

A total number of 707 studies were in the databases used. Through this search, a total of 15 studies that satisfied the criteria were identified as suitable for inclusion in this systematic review. The search followed PRISMA guidelines shown in Figure 1.

Study characteristics

The four research designs used in the included studies were all published between 2014 and 2023. Most of them took place in psychiatric care"(Aljumah et al., 2014; Warden et al., 2014; Aljumah & Hassali, 2015; Novick et al., 2015; Marasine et al., 2020; Chauhan et al., 2021; Teeng et al., 2021; Yusuf et al., 2021; Ruetsch et al., 2022),"and primary care"settings.(Burnett-Zeigler et al., 2014; Warden et al., 2014; Sirey et al., 2017; Wikberg et al., 2017)". Other studies carried out in the medical centre (Leggett et al., 2015), the research centre (Rossom et al., 2016), and community pharmacies (Shoji et al., 2023).

Out of fifteen studies, twelve of them used one adherence measure to assess antidepressant adherence except 2 studies "(Aljumah & Hassali, 2015; Ruetsch et al., 2022), and one study (Chauhan et al., 2021) used two and five adherence measures respectively. Furthermore, most research employed subjective measurements, except one study (Rossom et al., 2016) that solely utilized objective measures, and two studies used both adherence measures to assess antidepressant adherence "(Chauhan et al., 2021; Ruetsch et al., 2022)."

There are five studies focused on antidepressant adherence at the initiation phase"(Burnett-Zeigler et al., 2014; Aljumah & Hassali, 2015; Novick et al., 2015;

Sirey et al., 2017; Teeng et al.,2021)"while other five studies focused on the implementation phase"(Aljumah et al., 2014; Marasine et al., 2020; Chauhan et al., 2021; Yusuf et al., 2021; Ruetsch et al., 2022)."On the other hand, four out of fifteen studies assessed adherence at both initiation and implementation phase"(Leggett et al., 2015; Rossom et al., 2016; Wikberg et al., 2017; Shoji et al., 2023)."Table 1 provided a summary of the measures used in this systematic study. Table 2 showed the psychometric properties of adherence measures."

Risk of bias within studies

There are four study designs included in this review such as randomized clinical/controlled trial (RCT) (n = 5), quasi-experimental (n = 4), cohort (n = 2), and cross-sectional studies (n = 4). From all the 15 studies included in the review, 14 articles showed a high number of positive responses to the JBI tool's questions, indicating low risk of bias, while only one article had a moderate risk of bias. The evaluation of the included studies is shown in Figures 2a to 2d.

Adherence measures

Multiple adherence measurements have been utilized to assess the adherence of antidepressant medication. Self-report measures were frequently employed in research. The second most often used measure was the clinician-rating scale and pharmacy refill data, followed by the adherence scale, pill count and average serum level. Medication adherence was only evaluated in 14 studies during the initiation and/or implementation phase. There was no data about the adherence phase provided in 1 study (Warden et al., 2014). Moreover, no information on the antidepressants' discontinuation phase was presented in any of the included studies.

Psychometric properties of adherence measures

The psychometric characteristics of certain measures of adherence to antidepressant were assessed by employing the reliability and validity data. The predominant method employed to evaluate reliability was the utilization of Cronbach's alpha, which evaluates internal consistency. Meanwhile, the validity of the measures was evaluated by comparing them with Medication Event Monitoring Systems (MEMS) "(Burnett-Zeigler et al., 2014; Leggett et al., 2015; Sirey et al., 2017)."

Objective adherence measures

In this study, objective measures such as pharmacy refill data, pill count, and serum levels were utilized. Pharmacy refill data were used in two studies (Rossom et al., 2016; Ruetsch et al., 2022), and one study (Chauhan et al., 2021) used pill count and average serum levels. However, both studies did not report any psychometric properties. Antidepressant adherence was measured using clinic-based pill count where the carers were instructed to save and collect any medicine strips that patients had consumed over a period of three months. This measure often misclassified adherent patients as nonadherent, although it was effective at identifying nonadherence. Average level of mood stabilizer in plasma/serum level/ blood concentration of patients were also reported which showed the accuracy in determining nonadherence, with a relatively high capability to identify adherence.

Subjective adherence measures

Studies conducted between 2014 and 2023 consistently showed that self-reporting was the dominant and ongoing method used to subjectively assess adherence to antidepressants. Self-reports are tools that ask patients about their experiences using medications (Rickles et al., 2023). Some examples of self-reported assessments include the Brief Medication Questionnaire (BMQ), Morisky Medication Adherence Scale (MMAS), Drug Attitude Inventory, (DAI-10), clinician-rating scale and many more. However, this method may yield inaccurate results due to potential biases introduced by patients. For instance, patients may provide inaccurate information on questionnaires and diaries, or deliberately manipulate their medication intake by

Table 1*:* Summary of adherence measures.

No	Authors, year	Method to measure adherence			Reliability			Validity			Adherence phase
		Objective	Subjective	n	Provided data	Referred to other	No data	Provided data	Referred to other	No data	
1	Aljumah et al., 2014		/	1			/			/	Implementation
2	Burnett-Zeigler et al., 2014		/	1			/		/		Initiation
3	Warden et al., 2014		/	1			/			/	No data reported
4	Aljumah & Hassali, 2015		/	2		/				/	Initiation
			/			/				/	
5	Leggett et al., 2015		/	1			/		/		Initiation and implementation
6	Novick et al., 2015		/	1			/			/	Initiation
7	Rossom et al., 2016	/		1			/			/	Initiation and implementation
8	Sirey et al., 2017		/	1			/		/		Initiation
9	Wikberg et al., 2017		/	1			/			/	Initiation and implementation

10	Marasine et al., 2020		/	1	/					/	Implementation
11	Chauhan et al., 2021		/	5			/	/			Implementation
			/				/	/			
			/				/	/			
		/					/	/			
		/					/	/			
12	Teeng et al., 2021		/	1			/		/		Initiation
13	Yusuf et al., 2021		/	1		/			/		Implementation
14	Ruetsch et al., 2022	/		2			/			/	Implementation
			/				/			/	
15	Shoji et al., 2023		/	1	/					/	Initiation and implementation

n: Number of adherence measures.

Table 1: Psychometric evaluation of antidepressant adherence measures

Type of adherence measures			Reliability	Validity
Objective	**Subjective**	**Method**		
	Burnett-Zeigler et al., 2014 & Leggett et al., 2015	Brief Medication Questionnaire (by Svarstad)	*	Predictive"validity reported when comparing BMQ with dose omissions as measured by MEMS over a 7-day or 30-day period. (Svarstad et al., 1999)"
	Aljumah & Hassali, 2015	Morisky Medication Adherence Scale (MMAS)	Internal consistency (Cronbach's alpha): ● 0.61. (Morisky et al., 1986) ● 0.62. (Brown et al., 2005) ● 0.70. (Interian, 2010) ● 0.83.(Morisky et al., 2008)	*
		Beliefs about Medicine Questionnaire (by Horne)	Internal"consistency (Cronbach's alpha): ● 0.74 (specific-necessity beliefs), ● 0.63 (specific-concern beliefs), ● 0.73 (general-overuse beliefs), and ● 0.70 (general-harm beliefs). (Horne et al., 1999)	*
	Sirey et al., 2017	Brief Medication Questionnaire (BMQ by Svarstad)	*	Self-report measure validated against an electronic bottle cap data (MEMS Cap). (Svarstad et al., 1999)
	Marasine et al., 2020	Morisky Green Levine Adherence (MGLA) score	Internal consistency (Cronbach's alpha): ● 0.80	*
	Teeng et al., 2021	Brief Adherence Rating Scale (BARS)	*	Good sensitivity (73%) and specificity (74%).
	Yusuf et al., 2021	Medication Adherence	Good psychometric properties, and satisfactorily	Validated for use within the Nigerian

		Rating Scale (MARS)	predicts non-adherence.	setting.
	Chauhan et al., 2021	Morisky"Medication Adherence Questionnaire (MAQ)	*	• Specificity, "34-42%; PPVs, 40-44%; and LR negative, 0.70-0.96; indicate better at detecting adherence. • Sensitivity, 63-73%; NPVs, 54-70%; and LR positive, 1.02-1.16; indicate moderate ability to detect nonadherence."
		Drug Attitude Inventory (DAI-10)"	*	
		Compliance Rating Scale (CRS).	*	Good at sensitivity, but low specificity.
Chauhan et al., 2021		Clinic-based pill counts	*	
		Mood-stabiliser levels (Plasma/ Serum level/ blood conc.)	*	Moderately high specificity and PPVs combined with a"high sensitivity (88%) and higher accuracy (55%) in detecting nonadherence, together with a respectably strong capacity to detect adherence."
	Shoji et al., 2023	Drug Attitude Inventory (DAI)-10	The questionnaire's test-retest reliability and internal consistency has been proven for the Japanese translation.	*

* No data reported/available.

PPV:"positive predictive value.

LR: likelihood ratios.

NPV:"negative predictive value.

discarding tablets to create the appearance of adherence to the prescribed regimen.

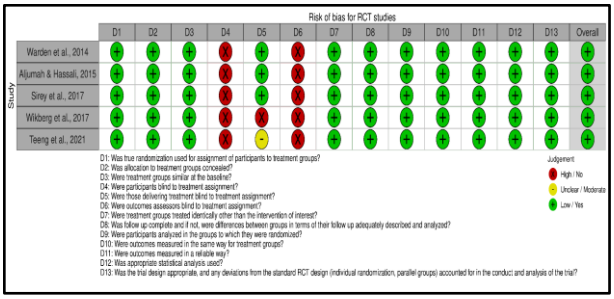

Fig. 2a: Risk of bias for RCT studies.

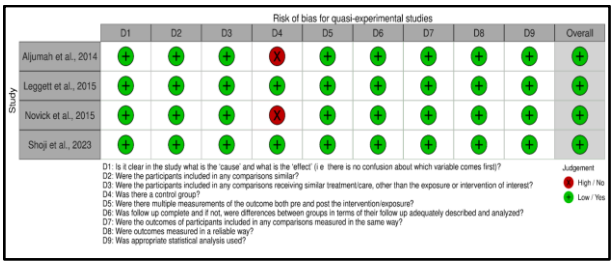

Fig. 2b: Risk of bias for quasi-experimental studies.

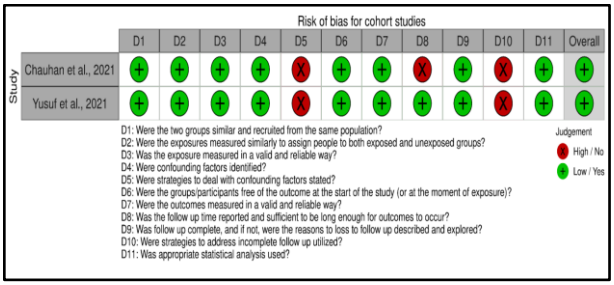

Fig. 2c: Risk of bias for cohort studies.

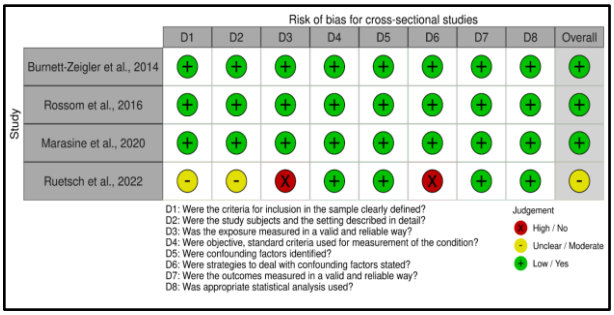

Fig. 2d: Risk of bias for cross-sectional studies.

Brief Medication Questionnaire (BMQ) (Svarstad et al., 1999)

The BMQ, developed by Svarstad, has two components. The first section has three primary components that inquire about patients' adherence to their prescription daily during the week before the interview. Additionally, it evaluates their perceptions of the effectiveness of the treatment and any adverse effects experienced. The second section has 11 questions that inquire about the challenges related to recalling medication-taking habits. It evaluates the obstacles related to physical and cognitive factors that affect adherence and self-confidence. The BMQ has been shown to be valid, with the regimen and belief screens having a sensitivity of 80-100% (Svarstad et al., 1999). Prior research has shown a substantial correlation between this measure and pharmacy refill data (Rickles and Svarstad, 2007). The review revealed that this method has been assessed in three studies (Burnett-Zeigler et al.,2014; Leggett et al., 2015; Sirey et al.,2017)."

Morisky Medication Adherence Scale (MMAS).

The MMAS is an effective screening tool for evaluating the usage of antidepressants. It contains eight items that assess behaviour and adherence to medication, designed to minimize any bias towards agreement. It has a total score ranging from 0 to 8, where higher numbers indicate greater adherence. This self-report questionnaire is a very reliable and validated tool developed by Morisky et al. "(Morisky et al., 1986; Morisky et al., 2008; Krousel-Wood et al., 2009; Morisky & DiMatteo, 2011)."It also has an Arabic version that has been made accessible (Alhalaiqa et al., 2011). The MMAS-8 was utilized in two studies (Aljumah et al., 2014; Aljumah & Hassali, 2015)."

It is a well-established tool for assessing adherence. Its reliability, as indicated by its Cronbach's alpha = 0.61, was reported in the initial research conducted on patients with hypertension "(Morisky et al.,1986)." The reliability of evaluating adherence to antidepressants, particularly during the

implementation period, has been demonstrated to be sufficient, with a Cronbach's alpha of 0.62"(Brown et al., 2005) and 0.70 (Interian, 2010)." This method demonstrates enhanced reliability and validity, especially in hypertension patients. The Cronbach's alpha = 0.83, while the sensitivity is 93%, and specificity is 53% were obtained after evaluating a specified time frame of 2 weeks "(Morisky et al., 2008)." Hence, the MMAS-8 demonstrates strong validity and has been empirically shown to be an effective screening method for assessing adherence to antidepressant treatment, particularly among Arabic patients.

Drug Attitude Inventory, 10-item version (DAI-10).

The DAI-10 questionnaire is a self-report scale consisting of ten items that is widely utilized for the purpose of assessing patients' attitudes regarding medication. Respondents select either true or false to answer each question. The scoring system assigns a value of 1 or −1 to each item, resulting in a total score that can range from −10 (indicating a very low attitude) to +10 (representing the finest possible attitude). Adherence is determined by a positive DAI-10 score, indicating a good subjective attitude, whereas a negative DAI-10 score indicates nonadherence with a poor subjective attitude. Higher scores on the DAI-10 indicate positive opinions towards drugs. This review includes two studies that examined the use of the DAI-10 scale to measure adherence to antidepressant "(Chauhan et al., 2021; Shoji et al., 2023)."

One study demonstrated the effectiveness of certain methods in assessing medication adherence "(Chauhan et al., 2021)." Specifically, they found that the Four-item Morisky Medication Adherence Questionnaire (MAQ) and the DAI-10 (with a higher cut-off) were more accurate in identifying adherence (with a specificity of 34-42%) compared to other measures. These methods also showed a moderate ability to identify nonadherence (sensitivity of 63-73%) when compared to the other measures. Meanwhile, other study has confirmed the internal consistency and test-retest reliability of the Japanese version of the DAI-10 questionnaire "(Shoji et al., 2023)."

Clinician-rating scale

The clinician-rating scale is a subjective assessment tool in which clinicians were asked to express their professional judgement about the patient's adherence to the prescribed antidepressant medication. In a study, the researchers utilized the Clinical Global Impression-Severity scale (CGI-S) (Novick et al., 2015). During this phase, patients were questioned about the consistency of their medication intake for Major Depressive Disorder (MDD) since their first appointment, with four possible answer options. Adherent patients were defined as those who selected option "1" and/or those who took a daily medicine dose within the range of 80% to 120% of the recommended dosage. Meanwhile, other research found that clinicians assessed the adherence of antidepressants using the Compliance Rating Scale (CRS), where higher scores indicate better adherence "(Chauhan et al., 2021)." The results of the study indicated that the CRS had a high level of sensitivity in detecting nonadherence, while demonstrating only moderate agreement with the MAQ.

Other subjective measures

In one study, self-report Adherence Questionnaire (AQ) was used where patients reported on their medication intake over the past 7 days, whether they followed the prescription or adjusted, and specified reasons for these actions "(Warden et al.,2014)." However, this method did not report about the validity or reliability. Next, another study used the Beliefs about Medicine Questionnaire (BMQ by Horne, 1999) to assess patients' medication-related beliefs "(Aljumah & Hassali, 2015)." It consists of two components, namely the BMQ-specific and the BMQ-general. The BMQ's internal consistency reliability has been assessed in patients with

psychiatric conditions.

The Brief Adherence Rating Scale (BARS) is a measure used by clinicians to evaluate adherence. It was described in one research "(Teeng et al., 2021)." The assessment consists of three questions and a visual analogue scale to measure the percentage of dosages taken in the previous month, ranging from 0% to 100%. The BARS assessment showed a high level of sensitivity "73%" and specificity "74%" in accurately identifying outpatients who were not adhering to their prescribed treatment regimen. The BARS used in this investigation was only validated for the administration of oral antipsychotics. In addition, patient self-report Beck Depression Inventory-II (BDI-II) was used to measure depression symptoms in a study "(Wikberg et al., 2017). Adherence to antidepressants is defined when BDI score ≤36 and have the (BDI <13) after 3-month follow-up. However, psychometric properties of this method were not reported.

Furthermore, one study used a Hindi version of the MAQ to assess self-reported adherence over a period of three months "(Aljumah & Hassali, 2015). Due to its well-established sensitivity and specificity in identifying non adherence, MAQ was chosen as the benchmark for the other measurements. Next, another study reported about the reliability of self-report Four-item Morisky Green Levine Adherence (MGLA) which the tool had a Cronbach's alpha of 0.80, indicating strong internal consistency (Marasine et al., 2020)."The MGLA score is a structured instrument consisting of four items. Each item requires a dichotomous answer (yes or no). A low degree of adherence is indicated by a score of 3 or 4, moderate adherence level score = 1 or 2, and high adherence level score = 0.

Yusuf et al. (2021) reported that the Medication Adherence Rating Scale (MARS) is a self-reporting tool consisting of 10 items. Each question requires a 'yes' or 'no' answer. The overall score on the MARS may vary from 0 to 10, with a higher score suggesting more adherence to medicine. The scale has strong psychometric qualities, effectively predicts non-adherence, and has been successfully validated for use in the Nigerian settings. Lastly, a study measures the adherence of antidepressants by using patient report, collateral report, and psychopathology for patients with Bipolar Depression (BD) or Major Depressive Disorder (MDD) "(Ruetsch et al., 2022)." However, no psychometric properties of this measure were reported.

Discussions

The utilization of an adherence measurement tool for adults with depression taking antidepressants holds significant importance in the overall management of the condition. Monitoring adherence provides crucial insights into whether patients are consistently following their prescribed medication regimen. This information is essential for healthcare professionals to assess treatment effectiveness, prevent relapses, and tailor therapeutic strategies, ultimately contributing to improved mental health outcomes and a better quality of life for individuals with depression. Regular use of adherence measurement tools enhances communication between patients and healthcare providers, fostering a collaborative approach in optimizing antidepressant therapy for the well-being of adult patients with depression.

This systematic review assesses the psychometric features of all measures of adherence used to analyse the behaviour of antidepressant consumption in people with depression. In this research, measurements of adherence were specifically focused on two phases: initiation and implementation. The discontinuation phase of pharmacotherapy was not reported. This review classified studies as being in the *"initiation phase of the adherence process"* if they recruited participants who had recently been prescribed antidepressant medications or if the study indicated that participants were starting therapy with antidepressant medications for the first time. Meanwhile, the implementation phase focused on participants who were currently taking the medication, and the discontinuation

phase focused on patients who had stopped taking the medication.

Self-report such as BMQ by Svarstad, pharmacy refill data, BDI-II, and DAI-10 have the capability to measure adherence at more than one adherence phase which can capture both initiation, and implementation of treatment (Leggett et al., 2015; Rossom et al., 2016; Wikberg et al., 2017; Shoji et al., 2023). However, studies by Burnett-Zeigler et al. (2014), and Sirey et al. (2017) capture BMQ by Svarstad on initiation phase only, while Chauhan et al. (2021) used DAI-10 only at implementation phase. The lack of comprehensive data from the recruited studies has made it challenging to determine the adherence phases.

Psychometric properties refer to the validity and reliability of the measurement tool (Asunta et al., 2019). Reliability that included the studies are test-retest reliability and internal consistency. Test-retest reliability pertains to the inherent stability of an assessment across time, evaluating the extent to which the scores obtained from the measuring instrument remain constant over successive test administrations (Berchtold, 2016). Internal consistency evaluates the extent to which the items in the questionnaire effectively measure the same underlying concept. Measures with a value of 0.80 or more are deemed excellent, while the least acceptable value for Cronbach's alpha is 0.7; however, values above 0.6 are also accepted (Griethuijsen et al., 2014; Taber, 2018). Furthermore, the included validity is specifically predictive validity. Confirming predictive validity involves providing evidence that the scale accurately predicts a gold-standard criteria that will be tested at a later point in time (Lazar et al., 2017).

The most frequently employed objective measure was pharmacy refill data (Rossom et al., 2016; Ruetsch et al., 2022), followed by pill count and average serum levels (Chauhan et al., 2021). However, another systematic review reported that MEMS is the predominant objective measure, with pharmacy records being the subsequent commonly utilized method to measure adherence in unipolar depression only. MEMS is widely acknowledged as a benchmark for adherence, often considered a "gold standard" (Srimongkon et al., 2019). Following this, while pill counts are frequently employed to assess adherence in bipolar disorder, their reliability is questionable due to uncertainty about whether the dispensed tablets are taken (Chauhan et al., 2021). Consequently, it is widely suggested that pill counts are only effective when conducted unexpectedly during home visits (Shiomi et al., 2021).

Moreover, average serum level is a direct method measuring the antidepressant or its metabolite concentration in a patient's blood or urine. This method is particularly useful for specific antidepressants that have measurable markers (Cristea et al., 2019). However, this method may not be suitable for certain drugs with extended half-lives that can still be detected in patients even after treatment ends (Anghel et al., 2019). For objective measure, only study by Chauhan et al. (2021) reported about the validity of the tool where it was revealed that pill counts were effective in detecting nonadherence (sensitivity) but were not reliable in detecting adherence (specificity). Additionally, the tool often incorrectly identified patients as non adherent. Although the yields are low, the levels of moodstabilizers in serum or plasma showed a high sensitivity (88%) and greater accuracy (55%) in detecting nonadherence. They also demonstrated a reasonably high ability to detect adherence, with moderately high specificity and positive predictive value.

Self-report measures were often favoured in adherence research and were the most used subjective measure of adherence at both initiation and/or implementation phase. This method may yield inaccurate results due to potential biases introduced by patients. For instance, patients may provide inaccurate information on questionnaires and diaries, or deliberately manipulate their medication intake by discarding tablets to create the appearance of adherence to the prescribed regimen (Anghel et al., 2019).

A commonly used self-report measure was the BMQ by Svarstad et al. Although Svarstad's BMQ psychometric properties have been investigated in other chronic illnesses, only minimal associations with pharmacy refill data have been found. The questionnaire's validity was confirmed by referring it to other data. It found predictive validity when comparing the questionnaire (BMQ) with dose omissions recorded by MEMS over 7-day or 30-day periods (Burnett-Zeigler et al., 2014; Leggett et al., 2015).

MMAS-8 was another self-report measure that was indicated for patients who are taking antidepressant medications for depression, as well as for other medical disorders. It demonstrated acceptable reliability (Cronbach's alpha = 0.61) in research in patients with hypertension, adequate reliability (Cronbach's alpha = 0.62 and 0.70) when assessing adherence to antidepressant medicines at the implementation phase of adherence (Aljumah & Hassali, 2015). This tool also showed better reliability and validity (Cronbach's alpha = 0.83, sensitivity = 93%, and specificity = 53%) albeit in hypertensive patients when considering a specific time frame of 2 weeks.

The BMQ by Horne et al. is another self-report measure that has acceptable internal consistency reliability with Cronbach's alpha = 0.74, 0.63, 0.73, and 0.70 for BMQ specific-necessity beliefs, specific-concern beliefs, general-overuse beliefs, and general-harm beliefs respectively when used as a measure of medication adherence in depression (Aljumah & Hassali, 2015). Studies by Marasine et al. (2020), and Yusuf et al. (2021) reported good reliability for the tools where MGLA score showed internal consistency (Cronbach's alpha = 0.80), and MARS demonstrated good psychometric properties, and satisfactorily predicts non-adherence for measuring adherence of antidepressants in patient with depression.

Out of 15 studies that included, only 9 studies reported the psychometric properties of adherence measures where 3 studies, 5 studies, and 1 study reported reliability, validity, and both properties respectively. It is worth mentioning that past research on medication adherence employed just one measure, but most recent studies now use several measures of adherence (Aljumah & Hassali, 2015; Chauhan et al., 2021; Ruetsch et al., 2022), acknowledging that various measures assess distinct elements of adherence. Since there is no universally accepted "*gold standard*" for adherence evaluation, opting for a dual strategy that incorporates both direct and indirect methods, as suggested by Jimmy and Jose (2020) and Srimongkon et al. (2019), can enhance the accuracy of results.

There are several limitations of this systematic review. Firstly, most of the studies analyzed in this review focused on medication adherence during the initiation and implementation phases. It is important to note that none of the studies specifically examined medication adherence at the discontinuation phase. This highlights a notable gap in the existing research, as there is no data available on the patients who have been prescribed antidepressant medication but have discontinued its use. Secondly, the systematic reviews require substantial time and resources. Hence, it can be challenging to conduct a comprehensive review within limited time frames or with limited resources.

Next, this study specifically examines individuals with depression who do not have any other comorbidity, with the aim of improving the reliability of the results. However, it is important to note that the the findings may be restricted to general population, as they may not apply to individuals with specific types of depression, comorbidity, or adolescents. Lastly, not all the included articles reported about the psychometric properties of the methods to measure medication adherence. This will affect the validity of data for the most reliable and validated method used to measure medication adherence.

Conclusion

In conclusion, the systematic review on the

assessment of methods to measure adherence of antidepressants in adults with depression reveals a utilization of both diverse range objective and subjective measures, particularly during the initiation and implementation adherence phases. Self-report assessments have become the most often used and convenient instruments in primary care and psychiatric settings when assessing antidepressant adherence in adults with depression. They are followed by clinician-rating scales and pharmacy refill data, adherence scales, pill counts, and average serum levels (biological markers). Although an assessment of psychometric properties was conducted, no single standard measure with consistently strong reliability and validity across different stages of adherence was found. This highlights the need of using a comprehensive strategy that incorporates both subjective and objective assessments. Considering the lack of a definitive benchmark as gold standard, it is advisable to use this practical method for evaluating compliance in individuals with depression.

Authors Contributions

The authors, N.A.A.G., H.A.A.M., and S.Z. contributed jointly to the research. Their collaborative efforts involved reviewing and performing literature synthesis to comprehensively assess the various methods must for measuring adherence to antidepressant medications in individuals with depression. Additionally, they provided recommendations for future research, the importance of combining subjective and objective measures for practical adherence assessment in this population need to be emphasize.

Conflict of interest

The authors declare no conflict of interests. This study conducted with transparency and impartiality to ensure the reliability and credibility of the systematic review.

References

Al-Jumah, K., Ahmad Hassali, A., & AlQhatani, S. (2014). Examining the relationship between adherence and satisfaction with antidepressant treatment. Neuropsychiatric Disease and Treatment, 2014:10, 1433–1438. https://doi.org/10.2147/ndt.s67008

Alhalaiqa, F., Deane, K. H. O., Nawafleh, A. H., Clark, A., & Gray, R. (2011). Adherence therapy for medication non-compliant patients with hypertension: a randomised controlled trial. Journal of Human Hypertension, 26(2), 117–126. https://doi.org/10.1038/jhh.2010.133

Aljumah, K., & Hassali, M. (2015). Impact of pharmacist intervention on adherence and measurable patient outcomes among depressed patients: a randomised controlled study. BMC Psychiatry, 15(1). https://doi.org/10.1186/s12888-015-0605-8

Anghel, L. A., Farcas, A. M., & Oprean, R. N. (2019). An overview of the common methods used to measure treatment adherence. Medicine and Pharmacy Reports, 92(2), 117–122. https://doi.org/10.15386/mpr-1201

Asunta, P., Viholainen, H., Ahonen, T., & Rintala, P. (2019). Psychometric properties of observational tools for identifying motor difficulties – a systematic review. BMC Pediatrics, 19(1). https://doi.org/10.1186/s12887-019-1657-6

Berchtold, A. (2016). Test–retest: Agreement or reliability? Methodological Innovations, 9(1), 205979911667287. https://doi.org/10.1177/2059799116672875

Brown, C., Battista, D. R., Bruehlman, R., Sereika, S. S., Thase, M. E., & Dunbar-Jacob, J. (2005). Beliefs About Antidepressant Medications in Primary Care Patients. Medical Care, 43(12), 1203–1207. https://doi.org/10.1097/01.mlr.0000185733.30697.f6

Burnett-Zeigler, I., Kim, H. M., Chiang, C., Kavanagh, J., Zivin, K., Rockefeller, K., Sirey, J. A., & Kales, H. C. (2013). The association between race and gender, treatment attitudes, and antidepressant treatment adherence. International Journal of Geriatric Psychiatry, 29(2), 169–177. https://doi.org/10.1002/gps.3984

Chand, S. P., & Arif, H. (2021). Depression. PubMed; StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK430847

Chauhan, N., Chakrabarti, S., & Grover, S. (2021). Identifying Poor Adherence in Outpatients with Bipolar Disorder: A Comparison of Different Measures. Journal of Neurosciences in Rural Practice, 13(01), 012–022. https://doi.org/10.1055/s-0041-1736155

Cristea, I. A., Karyotaki, E., Hollon, S. D., Cuijpers, P., & Gentili, C. (2019). Biological markers evaluated in randomized trials of psychological treatments for depression: a systematic review and meta-analysis. Neuroscience & Biobehavioral Reviews, 101, 32–44. https://doi.org/10.1016/j.neubiorev.2019.03.022

Dell'Osso, B., Albert, U., Carrà, G., Pompili, M., Nanni, M. G., Pasquini, M., Poloni, N., Raballo, A., Sambataro, F., Serafini, G., Viganò, C., Demyttenaere, K., McIntyre, R. S., & Fiorillo, A. (2020). How to improve adherence to antidepressant treatments in patients with major depression: a psychoeducational consensus checklist. Annals of General Psychiatry, 19(1). https://doi.org/10.1186/s12991-020-00306-2

Gast, A., & Mathes, T. (2019). Medication Adherence Influencing Factors—an (updated) Overview of Systematic Reviews. Systematic Reviews, 8(1). https://doi.org/10.1186/s13643-019-1014-8

Griethuijsen, R. A. L. F., van Eijck, M. W., Haste, H., den Brok, P. J., Skinner, N. C., Mansour, N., Savran Gencer, A., & BouJaoude, S. (2014). Global Patterns in Students' Views of Science and Interest in Science. Research in Science Education, 45(4), 581–603. https://doi.org/10.1007/s11165-014-9438-6

Horne, R., Weinman, J., & Hankins, M. (1999). The beliefs about medicines questionnaire: The development and evaluation of a new method for assessing the cognitive representation of medication. Psychology & Health, 14(1), 1–24. https://doi.org/10.1080/08870449908407311

Interian, A. (2010). A Brief Self-Report Measure to Assess Antidepressant Adherence Among Spanish-Speaking Latinos. Journal of Clinical Psychopharmacology, 30(6), 755–757. https://doi.org/10.1097/jcp.0b013e3181fb57f8

Jimmy, B., & Jose, J. (2020). Patient Medication Adherence: Measures in Daily Practice. Oman Medical Journal, 26(3), 155–159. https://doi.org/10.5001/omj.2011.38

Krousel-Wood, M., Islam, T., Webber, L. S., Ré, R. N., Morisky, D. E., & Muntner, P. (2009). New medication adherence scale versus pharmacy fill rates in seniors with hypertension. Am J Manag Care. 2009, 15(1), 59–66.

Lam, W. Y., & Fresco, P. (2015). Medication Adherence Measures: An Overview. BioMed Research International, 2015(217047), 1–12. https://doi.org/10.1155/2015/217047

Lazar, J., Feng, J. H., & Hochheiser, H. (2017). Analyzing qualitative data. Research Methods in Human Computer Interaction, 299–327. https://doi.org/10.1016/b978-0-12-805390-4.00011-x

Leggett, A., Kavanagh, J., Zivin, K., Chiang, C., Kim, H. M., & Kales, H. C. (2015). The Association Between Benzodiazepine Use and Depression Outcomes in Older Veterans. Journal of Geriatric Psychiatry and Neurology, 28(4), 281–287. https://doi.org/10.1177/0891988715598227

Marasine, N. R., Sankhi, S., Lamichhane, R., Marasini, N. R., & Dangi, N. B. (2020). Self-Reported Antidepressant Drug Side Effects, Medication Adherence, and Its Associated Factors among Patients Diagnosed with Depression at the Psychiatric Hospital of Nepal. Depression Research and Treatment, 2020, 1–7. https://doi.org/10.1155/2020/7024275

McGuinness, L. A., & Higgins, J. P. T. (2020). Risk-of-bias VISualization (robvis): An R package and Shiny web app for visualizing risk-of-bias assessments. Research Synthesis Methods, 12(1). https://doi.org/10.1002/jrsm.1411

Morisky, D. E., Green, L. W., & Levine, D. M. (1986). Concurrent and Predictive Validity of a Self-reported Measure of Medication Adherence. Medical Care, 24(1), 67–74. https://doi.org/10.1097/00005650-198601000-00007

Morisky, D. E., Ang, A., Krousel-Wood, M., & Ward, H. J. (2008). Predictive Validity of a Medication Adherence Measure in an Outpatient Setting. The Journal of Clinical Hypertension, 10(5), 348–354. https://doi.org/10.1111/j.1751-7176.2008.07572.x

Morisky, D. E., & DiMatteo, M. R. (2011). Improving the measurement of self-reported medication nonadherence: Response to Authors. Journal of Clinical Epidemiology, 64(3), 255–257. https://doi.org/10.1016/j.jclinepi.2010.09.002

Novick, D., Montgomery, W., Moneta, V., Peng, X., Brugnoli, R., & Haro, J. M. (2015). Antidepressant medication treatment patterns in Asian patients with major depressive disorder. Patient Preference and Adherence, 2015:9, 421–428. https://doi.org/10.2147/ppa.s68432

Ormel, J., Kessler, R. C., & Schoevers, R. (2019). Depression. Current Opinion in Psychiatry, 32(4), 348–354. https://doi.org/10.1097/yco.0000000000000505

Page, M. J., McKenzie, J. E., Bossuyt, P. M., Boutron, I., Hoffmann, T. C., Mulrow, C. D., Shamseer, L., Tetzlaff, J. M., Akl, E. A., Brennan, S. E., Chou, R., Glanville, J., Grimshaw, J. M., Hróbjartsson, A., Lalu, M. M., Li, T., Loder, E. W., Mayo-Wilson, E., McDonald, S., & McGuinness, L. A. (2021). The PRISMA 2020 statement: an Updated Guideline for Reporting

Systematic Reviews. British Medical Journal, 372(71). https://doi.org/10.1136/bmj.n71

Rickles, N. M., Mulrooney, M., Sobieraj, D., Hernandez, A. V., Manzey, L. L., Gouveia-Pisano, J. A., Townsend, K. A., Luder, H., Cappelleri, J. C., & Possidente, C. J. (2023). A systematic review of primary care-focused, self-reported medication adherence tools. Journal of the American Pharmacists Association: JAPhA, 63(2), 477-490.e1. https://doi.org/10.1016/j.japh.2022.09.007

Rossom, R. C., Shortreed, S., Coleman, K. J., Beck, A., Waitzfelder, B. E., Stewart, C., Ahmedani, B. K., Zeber, J. E., & Simon, G. E. (2016). Antidepressant Adherence across Diverse Populations and Healthcare Settings. Depression and Anxiety, 33(8), 765–774. https://doi.org/10.1002/da.22532

Ruetsch, C., Liberman, J., Davis, T., Sajatovic, M., Velligan, D., & Forma, F. (2022). The Effect of Objectively Collected Medication Adherence Information on Bipolar I and Major Depressive Disorder Treatment Decisions: A Randomized Case Vignette Study of Psychiatric Clinicians. Journal of Affective Disorders Reports, 100344. https://doi.org/10.1016/j.jadr.2022.100344

Sansone, R. A., & Sansone, L. A. (2019). Antidepressant adherence: are patients taking their medications? Innovations in Clinical Neuroscience, 9(5-6), 41–46. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3398686/

Shiomi, M., Kurobuchi, M., Tanaka, Y., Takada, T., & Otori, K. (2021). Pill Counting in the Determination of Factors Affecting Medication Adherence in Patients with Type 2 Diabetes: A Retrospective Observational Study. Diabetes Therapy, 12. https://doi.org/10.1007/s13300-021-01091-1

Shoji, M., Maeda, H., Watanabe, F., Kazunori Tanuma, Fujiwara, A., Iwanaga, Y., Shimada, A., & Onda, M. (2023). A non-randomized, controlled, interventional study to investigate the effects of community pharmacists' cognitive behavioral therapy–based interventions on medication adherence and relevant indicators in patients with depression. BMC Psychiatry, 23(1). https://doi.org/10.1186/s12888-023-04602-5

Sirey, J. A., Banerjee, S., Marino, P., Bruce, M. L., Halkett, A., Turnwald, M., Chiang, C., Liles, B., Artis, A., Blow, F., & Kales, H. C. (2017). Adherence to Depression Treatment in Primary Care. JAMA Psychiatry, 74(11), 1129. https://doi.org/10.1001/jamapsychiatry.2017.3047

Srimongkon, P., Aslani, P., & Chen, T. F. (2019). A systematic review of measures of medication adherence in consumers with unipolar depression. Research in Social and Administrative Pharmacy, 15(1), 3–22. https://doi.org/10.1016/j.sapharm.2018.02.008

Stewart, S.-J. F., Moon, Z., & Horne, R. (2022). Medication nonadherence: health impact, prevalence, correlates, and interventions. Psychology & Health, 1–40. https://doi.org/10.1080/08870446.2022.2144923

Svarstad, B. L., Chewning, B. A., Sleath, B. L., & Claesson, C. (1999). The brief medication questionnaire: A tool for screening patient adherence and barriers to

adherence. Patient Education and Counseling, 37(2), 113–124. https://doi.org/10.1016/s0738-3991(98)00107-4

Taber, K. S. (2018). The use of cronbach's alpha when developing and reporting research instruments in science education. Research in Science Education, 48(6), 1273–1296. https://doi.org/10.1007/s11165-016-9602-2

Teeng, L. P., Guan, N. C., Kadir, M. S., & Ling, T. S. (2021). Reminder through mobile messaging application improves outpatient attendance and medication adherence among patients with depression: An open-label randomised controlled trial. Medical Journal of Malaysia, 76(5), 617–623.

Vrijens, B., De Geest, S., Hughes, D. A., Przemyslaw, K., Demonceau, J., Ruppar, T., Dobbels, F., Fargher, E., Morrison, V., Lewek, P., Matyjaszczyk, M., Mshelia, C., Clyne, W., Aronson, J. K., & Urquhart, J. (2012). A new taxonomy for describing and defining adherence to medications. British Journal of Clinical Pharmacology, 73(5), 691–705. https://doi.org/10.1111/j.1365-2125.2012.04167.x

Warden, D., Trivedi, M. H., Carmody, T., Toups, M., Zisook, S., Lesser, I., Myers, A., Kurian, K. R. B., Morris, D., & John Rush, A. (2014). Adherence to Antidepressant Combinations and Monotherapy for Major Depressive Disorder. Journal of Psychiatric Practice, 20(2), 118–132. https://doi.org/10.1097/01.pra.0000445246.46424.fe

Wikberg, C., Westman, J., Petersson, E-L., Larsson, M. E. H., André, M., Eggertsen, R., Thorn, J., Ågren, H., & Björkelund, C. (2017). Use of a self-rating scale to monitor depression severity in recurrent GP consultations in primary care – does it really make a difference? A randomised controlled study. BMC Family Practice, 18(1). https://doi.org/10.1186/s12875-016-0578-9

Yusuf, H., Magaji, M. G., Maiha, B. B., Yakubu, S. I., Haruna, W. C., & Mohammed, S. (2021). Impact of pharmacist intervention on antidepressant medication adherence and disease severity in patients with major depressive disorder in fragile north-east Nigeria. Journal of Pharmaceutical Health Services Research, 12(2021). https://doi.org/10.1093/jphsr/rmab030

Journal of Pharmacy

In vitro kinetics characterisation of polymeric nanoparticles for anticancer therapy

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Abstract

World Health Organization (WHO) predicts that cancer incidence will increase in the future, thus research involving anticancer agents such as nanoparticles has gained significant importance. Nanoparticles can be made from various materials, but the focus on polymeric chitosan and/or carrageenan-based nanoparticles is significant. Research on these materials investigates dynamic parameters of *in vitro* drug release, stability under working conditions and stability under storage conditions (*in vitro* kinetics characterisations). Here, a literature review is conducted to provide in-depth insights on research methodology trends, drawbacks, suitability, suggestions for improvements and findings related to polymeric carrageenan and/or chitosan nanoparticles for anticancer therapy. Journal articles involving nanoparticles made from chitosan and/or carrageenan containing anticancer agents published between 2017 and 2022 were acquired through Google Scholar search using relevant keywords. Generally, the methods used to investigate drug release kinetics of nanoparticles can be categorised into dialysis membrane, sample and separate or direct measurement methods. Studies on the response of physiochemical characteristics towards changes in environment do not vary highly and are generalisable. Stability studies primarily measure the physicochemical changes of nanoparticles as a response measurement towards storage conditions. Both drug release selectivity and physicochemical characteristics response in different pH environments were found to be predictable *via* the ionisation of polymers and drugs used in different pH. The size of the nanoparticles formed during polyelectrolyte complexation process was found to be at its minimum at a balanced pH, possibly due to increased polymer-polymer attraction. The methods used for *in vitro* kinetics studies were generalised, and suggestions to address potential sources of errors were given in the current review. The selectivity of drug release and changes in physicochemical characteristics of the nanoparticles in different pH environments were found to largely coincide with the principles of ionisation of nanoparticle constituent.

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Introduction

The World Health Organization (WHO) has predicted that between 2008 and 2030, the incidence of cancer will increase at an alarming rate, which is a rate of 40% in high-income countries, and 80% in low-income countries. The organisation has also predicted that by the year 2030 the diagnosis of new cancer cases will reach 10-11 million (World Health Organization, 2012). In fact, from 2018 to 2040, in Malaysia alone, the yearly incidence of breast cancer and lung cancer is expected to increase by more than 70% and 100%, respectively (World Health Organization, 2020). Based on these two facts, it is expected that the demand for more efficacious and safer treatment options will increase dramatically in the upcoming years, both locally and internationally. While chemotherapy is an available treatment option, the main problem that comes with chemotherapy is the inherently unavoidable adverse effects and the emerging problem of chemotherapy-resistant cancer (Al-Samydai et al., 2019). Thus, it is important to further continue research on cancer to find new anticancer therapy improvements that are more efficacious and are minimally toxic to the human body.

Throughout recent years, nanoparticles have gained attention as possible solutions for the problems found in anticancer therapy. Nanoparticles are conventionally defined as materials that are produced at a size approximately between 1 nm and 1000 nm (Zielińska et al., 2020). Due to their properties, nanoparticles may help overcome several problems seen in conventional medicine, including in cancer medicine. Nanoparticles are beneficial due to their ability to ensure that the drugs remain in the body for a longer time. This is because instead of one bolus of drugs being administered directly into the body, nanoparticles that contain the drug will exhibit a sustained-release kinetics (Chu et al., 2019; Trousil et al., 2020). This in turn will cause an increase in virtual half-life, which thus results in prolonged presence and action in the human body. In truth, the actual degradation of the drug by the body is unchanged, but the degradation of the drug can only occur for the drugs that have been released from the nanoparticles. Therefore, the drugs that are degraded are simply replaced due to the slow drug-release kinetics (S. Wang et al., 2020). Nanoparticles also help in specific targeting of organs or sites intended for therapy. This can be achieved due to the inherent kinetics of the nanoparticles, such as by being engulfed by macrophages or adhesion due to positively-charge surface (Chu et al., 2019; Trousil et al., 2020), or due to environment-responsive nature of the nanoparticles such as magnetic or acidic stimuli (S. Wang et al., 2020; Zhang et al., 2019), or due to the incorporation of targeting moieties such as ligands (Hoshyar et al., 2016). The selectivity of the nanoparticles implies that they may enhance the pharmacodynamics effect of the drug or reduce toxicity (Chu et al., 2019; Trousil et al., 2020; S. Wang et al., 2020; Zhang et al., 2019). This can be explained by the higher proportion of drugs being sequestered and released at the site of interest rather than at other sites of the body, and hence the effect of the formulation causes more therapeutic effect and fewer side effects.

Out of all the materials that have been used in nanomedicine, hydrogel shows a very promising characteristic. The biggest reason was due to its exceptional ability to protect drugs from enzymatic degradation (Utreja et al., 2020). It is also a stable nanoparticle due to the avoidance of coalescence and reduced drug leakage (Kharkwal & Janaswamy, 2017). Lastly, its ability to hold water and swell or shrink to control the rate of drug delivery and its biocompatible characteristics have caused it to gain significant traction for use in medicine (Narayanaswamy & Torchilin, 2019). Chitosan and carrageenan are two of the materials that have been used to formulate the nanoparticles and are extensively studied.

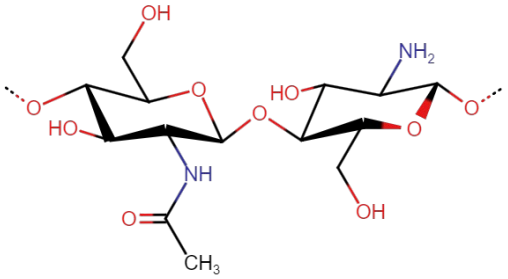

Fig. 1: The chemical structure of a chitosan monomer, which can either be in acetylated form (left) or deacetylated form (right).

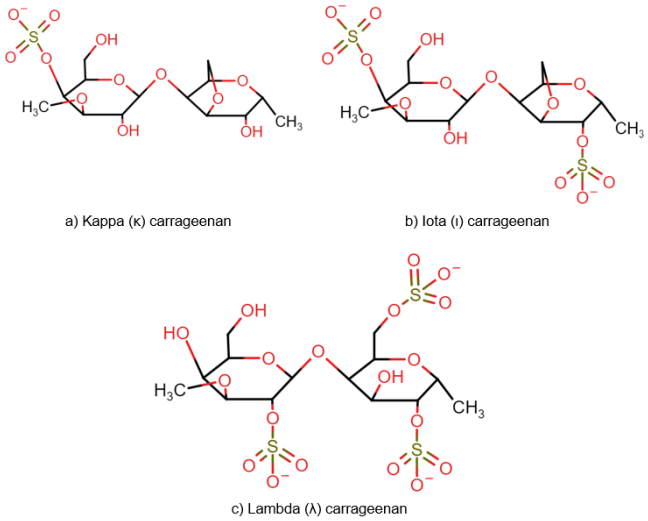

Fig. 2: The chemical structure of a carrageenan monomer. The different form of its (a) kappa, (b) iota and (c) lambda forms are delineated.

Chitosan is a polymer produced when naturally-occurring chitin undergoes deacetylation. The molecular structure consists of repeating chain units of N-acetyl-d-glucosamine and D-glucosamine, with each monomer unit having hydroxyl and amine groups (Rostami, 2020), as seen in Figure 1. Its popularity in nanomedicine can be attributed to its biocompatible, biodegradable, non-toxic and bioactive properties. To some extent, the positively charged chitosan may also impart site-specific targeting properties to chitosan nanoformulations (Ali & Ahmed, 2018). Meanwhile, carrageenan is an anionic polymer extracted from red algae that is used in pharmaceuticals, cosmetics and the food industry due to its gelling and thickening properties as well as its intrinsic antiviral, immune-activating and anticoagulant properties. Its molecular structure consists of polymer chains with alternating monomers of D-galactose and 3, 6-anhydro-galactose (Zia et al., 2017), which can be seen in Figure 2. Out of the three forms of carrageenan, only kappa (κ) and iota (ι) forms have considerable gel-forming properties, with the kappa(κ)-formed gels having a more strong and brittle properties while iota(ι)-formed gels having a more elastic and soft properties (Hotchkiss et al., 2016; Zia et al., 2017).

In the research of nanomedicine for cancer therapy involving these two polymers, the scheme of the study generally involves formulating the nanoparticle, followed by measurement of the static characteristics such as size, polydispersity index (PDI) and surface charge or zeta potential. This is followed by the measurement of dynamic characteristics such as drug release over time, stability under working conditions and stability under storage conditions. While the methodology of the first part of the study (i.e. static characteristics) has been widely researched and optimised, the second part of the study (i.e. dynamic characteristics) are highly variable in terms of their method due to differences in expected applications, objectives, focus, and understanding of the mechanics involved. Hence, there are considerable problems in regard to standardisation which therefore brings the question of accuracy, repeatability and suitability of methodology. Here, a literature review of the procedure used to measure drug release studies, stability under working conditions and stability under storage conditions in recent studies involving chitosan/carrageenan nanoparticles for anticancer therapy was conducted. In this literature review, the methods used in the measurement of dynamic characteristics were generalised, and the drawbacks and suitability of the decisions made in regard to study procedure were defined. Suggestions for consideration in future studies were also given in order to possibly improve research outcomes. Lastly, the findings of the research included in the literature

review were generalised to allow future researchers to predict the findings of their formulation more accurately.

Methodology

The current literature review involves the usage of search engines on the internet, which includes Google and Google Scholar. Our method involves two phases. The first phase (Phase I) is the acquisition and collection of journal publications and research accessible via open access or institutional access that will form the basic framework of current research practice and general key findings. In this phase, the criteria are that the articles collected must not be a review, must be a study involving nanoparticles or microparticles made from either chitosan, carrageenan and/or their derivatives, must contain substances with reported anticancer properties, and must be published within the previous 5 years (i.e. 2017-2022). The keywords used for this phase are chitosan, carrageenan, nanoparticles and anticancer.

Microparticles are included in the current phase of the literature review because, while nanoparticles have a higher propensity to be absorbed intact into cancer cells, microparticles share much of the same advantages with the nanoparticles. Primarily, it addresses the protection of drug degradation, overcoming the limitation of limited bioavailability, and selectivity in delivery is still possible when the choice of the polymer used is optimised. Macro-hydrogels are excluded due to the material being too different in physical nature from nanoparticles. Review articles are also excluded from Phase I of literature acquisition.

The second phase (Phase II) involves the acquisition of publications that are supplementary to the critiques, comments and suggestions given for the current research trend and serve as evidence that certain considerations must be made in future studies. For this phase, the usage of reviews, case studies, book publications and the like are included, which may or may not be focused on carrageenan, chitosan, nanoparticles and anticancer compounds but nevertheless are relevant considerations to the discussion at hand. The requirement for publication date for this phase is less stringent, although newer studies are prioritised and publications that are more than 5 years old are included if and only if publications relevant to the current discussion are not found.

Drug Release Profiling

General procedure and considerations in drug release

Drug release studies are conducted by allowing the nanoparticles to be exposed to the drug release media which mimics the physiological conditions of the body, while a predetermined volume of samples is taken at appropriate time intervals and replaced with an equal volume of drug release media. The samples are then analysed for drug content using spectrophotometric methods such as UV-Vis spectrophotometry or HPLC. The kinetics of the drug release is then graphed and, in some cases, analysed to fit onto zero order (Gaur et al., 2022; Irani & Nodeh, 2022; Nguyen et al., 2022; Sabra et al., 2018; Shafiee et al., 2019; Yan et al., 2018), first order (Gaur et al., 2022; Nguyen et al., 2022; Sabra et al., 2018; Shafiee et al., 2019; Yan et al., 2018), Higuchi (Gaur et al., 2022; Irani & Nodeh, 2022; Nguyen et al., 2022; Sabra et al., 2018; Sahu et al., 2017; Shafiee et al., 2019; Yan et al., 2018), Hixson Krowell (Gaur et al., 2022; Nguyen et al., 2022; Shafiee et al., 2019) and Korsmeyer Peppas (Gaur et al., 2022; Irani & Nodeh, 2022; Nguyen et al., 2022; Sabra et al., 2018) kinetics of drug release in order to postulate regarding the release mechanism of the drugs that are encapsulated. Drug release methods between studies typically differ in the methods of nanoparticle separation prior to analysis which may be classified into three; (a) dialysis membrane method, (b) sample and separate method which is the most widely used, and (c) direct measurement method which is cited in one study. The studies also differ in terms of the drug release media used, the drug release study period, volume of media used and less commonly, temperature.

The drug release media used is typically a saline buffer system, usually phosphate buffer, which has been adjusted to a certain pH which mimics the environment that nanoparticles are subjected to. This includes normal extracellular

conditions of pH 7.4 (Dhavale et al., 2021; Herdiana et al., 2022; Irani & Nodeh, 2022; Nogueira et al., 2020), cancer cell environment of pH 5.0-6.0 (Herdiana et al., 2022; Nogueira et al., 2020), cell cancer endosome of pH 4.0-5.5 (Dhavale et al., 2021; Herdiana et al., 2022; Nogueira et al., 2020; Vinothini et al., 2019), gastric conditions of pH 1.2 (Nguyen et al., 2022; Sabra et al., 2018, 2019; Sun et al., 2020; Yan et al., 2018; Yusefi et al., 2021), intestinal or colonic conditions with pH ranging between 4.50 to 7.40 (Nguyen et al., 2022; Sabra et al., 2018, 2019; Shafiee et al., 2019; Sun et al., 2020; Yan et al., 2018; Yusefi et al., 2021) as well as skin pH ranging from pH 5.0-7.4 (Sahu et al., 2017).

However, drug release media are not only limited to a simple buffer system. Some studies opted for a more complex media. In some studies, the buffer system used was supplemented with pepsin or pancreatin and bile to simulate gastric and intestinal conditions respectively (K. Liu et al., 2020; Sun et al., 2020). In some cases, the drug release media used was prepared from harvested fluids from animals, such as in the case of a study which harvested caecal contents from rats' caecum directly to be used for drug release studies (Sabra et al., 2018, 2019). This approach in preparation of drug release media, while more costly, comes with the advantage of being able to more accurately reflect the physiological conditions which the nanoparticles may be exposed to. The importance of taking into account the effect of enzymes and/or biological constituents in drug release studies should not be underestimated. Chitosan, on top of undergoing slow-rate non-enzymatic hydrolysis, also undergoes enzymatic hydrolysis in the human body (Jennings, 2017). Meanwhile, bile salts secreted in the intestinal phase of digestion may help with dissolution of hydrophobic drugs (Bourbon et al., 2018). Thus, wherever possible, relevant enzymes should be included in the drug release media.

For nanoparticles prepared for oral ingestion, the nanoparticles are expected to undergo several different conditions at different segments of the digestive tract. Because of this, the approach to the drug release studies might be different compared to formulations meant for other modes of administration. Typically, this would be investigated by carrying out the procedure at different pH levels as different "runs" of the procedure (Nguyen et al., 2022; Shafiee et al., 2019; Yan et al., 2018; Yusefi et al., 2021). However, a more accurate approach would be that the nanoparticles should be exposed to gastric conditions first prior to being introduced to intestinal conditions. This is because such an approach would take into account the possibility of non-reversible changes of the nanoparticle caused by gastric conditions prior to intestinal conditions (C. Liu et al., 2020). Fortunately, some studies have shown such an approach. In some studies involving nanoparticles meant for oral administration, the nanoparticles were suspended in a media with a pH of 1-2 for up to 2 hours before the nanoparticles were added into media resembling the intestinal system (K. Liu et al., 2020; Sabra et al., 2018; Sun et al., 2020). While procedures from one study to another might slightly differ, there exists an international consensus on how to conduct simulated digestion (Mulet-Cabero et al., 2020) which has been used as a guideline where formulations are expected to pass through the gastrointestinal tract.

The temperature used to study the drug release predominantly cites the usage of 37°C as the temperature condition used due to this temperature being physiologically relevant (Arif et al., 2017; Fan et al., 2020; Gaur et al., 2022; Herdiana et al., 2022; Irani & Nodeh, 2022; Ji et al., 2017; K. Liu et al., 2020; Nguyen et al., 2022; Nogueira et al., 2020; Sabra et al., 2018; Sahu et al., 2017; Shafiee et al., 2019; Sun et al., 2020; Yan et al., 2018; Yusefi et al., 2021). The temperature of 40°C has also been cited in one study (Shafiee et al., 2019), which may be relevant in applications involving induced hyperthermia for the purpose of selective delivery (Tharkar et al., 2019). However, some studies cited the usage of other temperatures, such as 26°C (Karimi et al., 2018; Mahdavinia et al., 2017), of which the reasoning behind such a parameter is unclear.

The drug release study period also varies, but it usually is conducted for at least 24 hours (Fan et al., 2020; Sabra et al., 2018, 2019). However, shorter periods may also be possible at 4-6 hours for gastrointestinal applications (K. Liu et al., 2020;

Nguyen et al., 2022; Sun et al., 2020). Longer study periods are also reported in literature, among the longest of which included in current literature review is 96 hours (Sahu et al., 2017) and 120 hours (Shafiee et al., 2019). In one study, drug release investigation was conducted without a predefined period, and instead was conducted until complete release of drug (Irani & Nodeh, 2022). However, there are some studies which have opted for drug release studies shorter than 24 hours without intention for oral application due to complete release of drug (Gaur et al., 2022; Herdiana et al., 2022; Mahdavinia et al., 2017).

To date, the importance of assessing drug release of free or unencapsulated drugs still remains unappreciated, in which many studies were found to not have carried out this assessment as a form of control. Degradation of drugs at physiological conditions are not to be underestimated, as it has been found that such observation may be significant (Abouelmagd et al., 2015; Bourbon et al., 2018; C. Liu et al., 2020; K. Liu et al., 2020; Moradi et al., 2021). Here, it should be highlighted that drug release studies involving only the drug-loaded nanoparticles may become questionable, as degradation of drugs, if significant, may artificially cause an appearance of slow drug release. In the same extension, the sustained release potential of nanoparticles may be underestimated if the nanoparticles give some sort of protection from drug degradation such as shown in a study (Moradi et al., 2021). Other than that, the assessment of drug release of unencapsulated drugs may also give more insight into the validity of the drug release method chosen.

The volume of the drug release media is equally important to be considered. Generally, sink conditions of at least 3 times the volume required to achieve saturated concentration of drug must be achieved (Abouelmagd et al., 2015). This is because violation of sink conditions leads to the inaccurate appearance of low drug release (Yu et al., 2019). While this is easily achieved for hydrophilic drugs, problems arise with hydrophobic drugs. This is because for this class of drugs, they would conventionally require a high volume of water, leading to analytical difficulty due to low drug concentration. Alternatively, samples may be concentrated after sampling, or drug release media may be added with a dissolution aid such as detergents which increase solubility of drug (Abouelmagd et al., 2015).

By and large, it is somewhat complicated to assess whether or not the majority of studies meet the volume requirement stated above. This is because not all studies report their procedure to the full extent, and the exact saturated solubility in physiological conditions would be expected to be different than that reported with pure water at 25°C, which is more available. However, generally, reports of saturated solubility studies in studies which encapsulates hydrophobic drugs, such as mangostin and curcumin, are rather scarce. Nonetheless, the volume of media varies highly from as low as 10 mL in a sample and separate method (Nogueira et al., 2020), and as high as 500 mL (Irani & Nodeh, 2022). Most studies cited the volume between 20-60 mL to be used as a drug release media (Herdiana et al., 2022; Ji et al., 2017; Karimi et al., 2018; Mahdavinia et al., 2017; Sahatsapan et al., 2021; Sahu et al., 2017; Sun et al., 2020; Yan et al., 2018).

Here, a required validation step should be considered which may help address some uncertainties associated earlier. A drug solubility experiment may be set-up whereby excess drug is allowed to incubate and agitate in the media chosen for drug release at conditions meant to be studied (ie. PBS, 100 rpm at 37°C), at two time points (such as at 7 hours, and 24 hours). Then, the media are to be sampled, centrifuged to separate solid undissolved drug, and its supernatant is spectrophotometrically analysed for drug concentration. This will allow experimental quantification of saturated concentration, and hence, allow the determination of volume which meets sink condition. Additionally, drug degradation in the media can be assessed by comparing the drug content detected at the two different time points (Abouelmagd et al., 2015). Alternatively, confirmation of sink condition may also be partially proven by including free drug in the drug release studies in similar amounts to encapsulated drugs. To summarise this section, suggestions are tabulated as shown in Table 1.

Table 1: Aspects of drug release profiling studies and suggestions to improve quality of data acquired.

Aspect	Suggestions	Remarks	References
The drug release media used	Simple buffer system fixed at certain pH	Drug release only affected by ionic and pH conditions	(Dhavale et al., 2021; Herdiana et al., 2022; Irani & Nodeh, 2022; Nguyen et al., 2022; Nogueira et al., 2020; Sabra et al., 2018, 2019; Sahu et al., 2017; Shafiee et al., 2019; Sun et al., 2020; Vinothini et al., 2019; Yan et al., 2018; Yusefi et al., 2021)
	Media supplemented by enzymes and biological constituent	Takes into account drug release caused by enzymatic degradation	(Bourbon et al., 2018; Jennings, 2017; K. Liu et al., 2020; Sun et al., 2020)
	Drug release media harvested directly from animal	Takes into account participation of constituents that may be present in the micro-scale	(Sabra et al., 2018, 2019)
Temperature of the drug release media throughout the procedure	37 °C	Results are more applicable/imposable for physiological therapy compared to other temperatures	(Arif et al., 2017; Fan et al., 2020; Gaur et al., 2022; Herdiana et al., 2022; Irani & Nodeh, 2022; Ji et al., 2017; K. Liu et al., 2020; Nguyen et al., 2022; Nogueira et al., 2020; Sabra et al., 2018; Sahu et al., 2017; Shafiee et al., 2019; Sun et al., 2020; Yan et al., 2018; Yusefi et al., 2021)
	40 °C (locally-induced hyperthermia)	May take into account thermal sensitivity which may be present in the nanoparticle system	(Shafiee et al., 2019)
Approach for orally-administered nanoparticles, requiring considerations of release profiles at different conditions; gastric, intestinal and colonic	Different batches of prepared nanoparticles are incubated at different conditions representing different segments of digestive tract throughout the whole study	The behaviour of nanoparticles' drug release profile at different conditions are considered separately – theoretical pH-release explanation may be more generalised	(Nguyen et al., 2022; Shafiee et al., 2019; Yan et al., 2018; Yusefi et al., 2021)
	Change in condition are simulated in sequence; gastric conditions, followed by intestinal conditions	Takes into account the nanoparticle matrix degradation caused by gastric conditions prior to exposure to intestinal or colonic conditions	(C. Liu et al., 2020; K. Liu et al., 2020; Mulet-Cabero et al., 2020; Sabra et al., 2018; Sun et al., 2020)

Study period	24 hours	The standard study period for nanoparticles meant for systemic circulation	(Fan et al., 2020; Sabra et al., 2018, 2019)
	>24 hours	May be suitable for nanoparticles with a really slow drug release	(Sahu et al., 2017; Shafiee et al., 2019)
	<24 hours	The study does not need to be prolonged more than needed in cases where all of the drugs has been released	(Gaur et al., 2022; Herdiana et al., 2022; Irani & Nodeh, 2022; Mahdavinia et al., 2017)
	4-6 hours	The standard study period for oral administration	(K. Liu et al., 2020; Nguyen et al., 2022; Sun et al., 2020)
Inclusion of free drug in the study as a means of control	Free drug needs to be included in the study	• Verifies that the results are not affected by degradation of drugs • May prove the sink conditions of the volume of media used • Able to take into account interference caused by nanoparticle separation methods	(Abouelmagd et al., 2015; Bourbon et al., 2018; C. Liu et al., 2020; K. Liu et al., 2020; Moradi et al., 2021)
Hydrophobic drug requiring high volume of media to achieve sink conditions and causing analytical difficulties due to highly diluted concentration	Concentrate the sample by means of evaporation prior to analysis	• Allows the usage of media without the presence of dissolution aid	(Abouelmagd et al., 2015)
	Add dissolution aid such as detergents	• Allows sink conditions to be achieved at low volume	(Abouelmagd et al., 2015)
Validation of sink conditions	Conduct drug solubility experiment in intended drug release media at relevant conditions (37°C etc.) for at least 24 hours	• Able to confirm that sink conditions (3 times the saturated volume) are achieved • Able to confirm that slow drug release is not due to saturation of media	(Abouelmagd et al., 2015)
	Inclusion of free drugs as one of the study groups.	• Partially proves that sink conditions are achieved	(Abouelmagd et al., 2015; Bourbon et al., 2018; C. Liu et al., 2020; K. Liu et al., 2020; Moradi et al., 2021)
Drug degradation during experiment period	Conduct drug solubility experiment in intended drug release media at relevant conditions (37°C etc.) and measured at two different time points	• Able to confirm that drug degradation is not significant factor in the study • Able to confirm that appearance of slow drug release is not caused by drug degradation	(Abouelmagd et al., 2015; Bourbon et al., 2018; C. Liu et al., 2020; K. Liu et al., 2020; Moradi et al., 2021)

Method to separate nanoparticles/adjust quantification to nanoparticle interference

As mentioned previously, the methods of drug release studies can be generally classified into three, which are the dialysis membrane method, the sample and separate method, and the direct measurement method. Of these three, the dialysis membrane method is the popular choice, followed by the sample and separate method, and then the direct measurement method. Only one study cites the direct measurement method, and it does not seem to be a widely-used method for quantification of drugs, presumably due to the questionable methodology and high uncertainty in regards to accuracy.

Dialysis membrane method

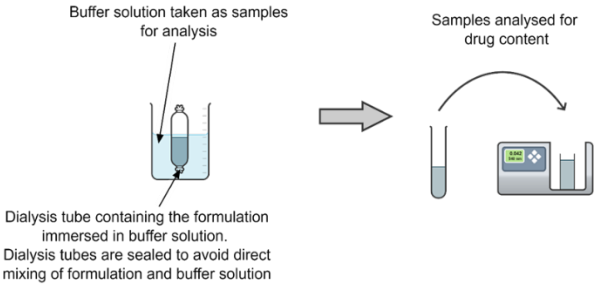

Fig. 3: The schematic diagram representing the dialysis membrane method of drug release studies.

In the dialysis membrane method, nanoparticles are put inside a dialysis bag which is then sealed and incubated in a drug release media. To this effect, the nanoparticles inside the dialysis bag are not mixed with the drug release media outside the dialysis bag. Instead, the drug has to be released inside the bag first before it can diffuse out as a free drug due to size selectivity of the membrane. This allows samples to be taken out and directly analysed without needing any particulate separation steps (Sahatsapan et al., 2021). A schematic diagram representing the dialysis membrane method is shown in **Figure 3**.

For nanoparticles that are dried, they are generally pre-dispersed in their respective drug release media prior to conducting the drug release studies (Fan et al., 2020; Gaur et al., 2022; Herdiana et al., 2022; Ji et al., 2017; Sahatsapan et al., 2021; Yan et al., 2018; Yusefi et al., 2021). For nanoparticles that are already pre-dispersed, they are generally allowed to retain their original dispersant when loaded into the dialysis membrane (Arif et al., 2017; K. Liu et al., 2020; Sahu et al., 2017; Vinothini et al., 2019). Generally, the dialysis bag is expected to be fully immersed into the drug release media. For buoyant dialysis tubes, generally, the help of a sinker may aid in ascertaining this condition (Gaur et al., 2022).

One of the strengths of the dialysis membrane method is that the separation step does not need to be carried out in this method (Modi & Anderson, 2013). This is not only more convenient, but also avoids the pitfalls related to the separation procedure that is inherent to separation methods. That is, the pressure applied in the sample and separate method inherently disturbs the equilibrium, and incomplete separations may cause data to be significantly erroneous (Modi & Anderson, 2013).

However, the usage of the dialysis tubes has its weaknesses. Mainly, those who opt to use this method are advised to properly consider the possibility of erroneous conclusion arising from the effect of the compartmentalisation of the donor phase (inside the dialysis tube) from the receiver/acceptor phase (outside the dialysis tube). It has been shown that wrongful interpretation of data can arise from the diffusion of drug through the dialysis membrane itself being a more significant rate-limiting step than the drug release from the nanoparticle, or that interactions of released drug with concentrated nanoparticle constituents significantly affecting the drug content in the donor compartment (Modi & Anderson, 2013; Moreno-Bautista & Tam, 2011; Wallace et al., 2012; Weng et al., 2020; Yu et al., 2019; Zambito et al., 2012).

To this extent, a few key decisions have to be made prior to conducting this method of study. The size selectivity of dialysis bags is often expressed as molecular weight cut-off (termed MWCO) which describes the size of the pore. While it is possible for drugs to diffuse through a

dialysis bag as long as the drug molecular weight is lower than the MWCO of the dialysis bag, it has been shown that selecting a dialysis membrane with a low MWCO may cause a lower rate of drug diffusion across the membrane despite being higher than the drug's molecular weight (Moreno-Bautista & Tam, 2011; Yu et al., 2019).

Additionally, drug diffusion across the membrane is also governed by the material of the membrane itself. While it has been suggested that the usage of membranes with 100 times higher MWCO than the size of the molecule would negate membrane resistance, a study demonstrated that a cellulose ester membrane which meets this criterion has a slower diffusion rate of doxorubicin compared to a regenerated cellulose membrane which does not meet this criterion (Yu et al., 2019). There has also been evidence suggesting that drug release may be overestimated due to destabilisation of the drug release membrane in acidic conditions which reduces efficient compartmentalisation of acceptor and donor phases of the experiment, and the interaction of the dialysis membrane with the nanoparticle, altering drug release (Weng et al., 2020).

Lastly, there have also been questions raised regarding using detergent-containing media for hydrophobic drugs. Particularly, most of the time, detergents could not pass through the dialysis membrane. Thus, the presence of detergent inside the dialysis bag may lead to the drug released to interact with the detergents, which in turn prevents the drug from passing through the dialysis bag. Meanwhile, if the detergent is only present outside the dialysis bag, then the drug may precipitate in the dialysis bag once it is released out of the nanoparticle, owing to their low solubility in water (Abouelmagd et al., 2015).

Here, to reduce the resistance of diffusion caused by the dialysis bag, dialysis tubes with higher MWCO should be selected. The study mentioned earlier has recommended that dialysis membranes with MWCO of 1000 kDa should be sufficiently small to provide a barrier to nanoparticles with a 100 nm size range. Additionally, as a means of validation, drug release from free drug and empty nanoparticles spiked with drug may be carried out to eliminate the possibility of misinterpretation due to the rate-limiting process of diffusion through the dialysis membrane, and the binding effect of the nanoparticle. Alternatively, instead of empty nanoparticles spiked with drugs, the study also suggested that repetition of the drug release studies at different drug-loaded nanoparticle concentrations may give additional insight (Modi & Anderson, 2013). Additionally, the possibility of using mathematical models proposed and investigated in one study may also be possible, although the usage of the model is not yet widely explored (Yu et al., 2019).

Sample and separate method

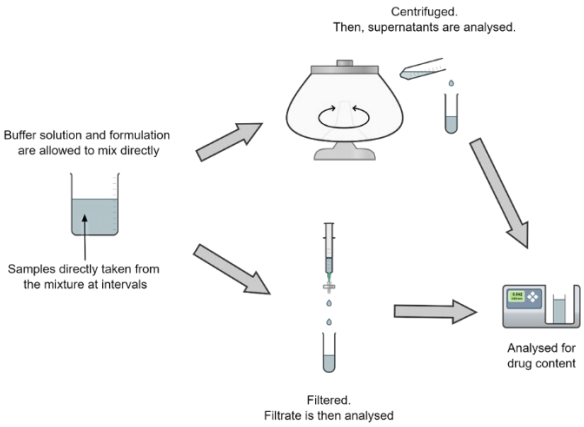

Fig. 4: The schematic diagram representing the dialysis membrane method of drug release studies.

Unlike the dialysis membrane method, this method of quantification involves allowing the nanoparticles to be directly dispersed in the drug release media in one compartment. Separation is then conducted *via* centrifugation (Mahdavinia et al., 2017; Sabra et al., 2018; Sun et al., 2020), magnetic separation (Nogueira et al., 2020), filtration, or combination, and the supernatant or filtrate are then analysed for drug content. A schematic diagram representing the sample and separate method is shown in **Figure 4**.

Unconventionally, due to the nature of this method, the drug release may be conducted either in a discontinuous method. In the continuous or conventional method, nanoparticles are suspended in drug released media. Then, the media are sampled at predetermined time points while allowing the nanoparticles to continuously release drugs without any discontinuation. The samples are put through a separation process prior to measurement (Mahdavinia et al., 2017; Sun et al., 2020). In the discontinuous method, the nanoparticles are suspended in release media and then at predetermined time points, all of the drug release media are put through the separation process. The supernatants are analysed and the pelleted nanoparticles are then resuspended in drug release media to recontinue the drug release investigation. With this approach, lower volumes of release media are required and thus concerns about excessive dilution may be addressed (Sabra et al., 2018).

Under the current scope, no filtration-based separation was chosen as the separation method. Instead, the centrifugation method was always chosen as a separation method, and all of the conditions of separation are relatively mild. Centrifugation speed only ranges from 4000-8000 rpm, for at most 10 minutes in all of the studies that fall under this category (Mahdavinia et al., 2017; Sabra et al., 2018; Sun et al., 2020). Nevertheless, in the upcoming section, widely recognised separation methods will be discussed. The goal is to provide future researchers with insights into the strengths and weaknesses of these methods, enabling them to make well-informed decisions.

The strength of this method is that drug release into the release media is not affected by the presence of a dialysis tube. As discussed previously under the dialysis membrane method, the dialysis rate may become the rate limiting step that causes an artificial appearance of slow release. Meanwhile, using the sample and separate method, such a weakness is not seen (Wallace et al., 2012). Centrifugation steps also seem to address the possibility of drug adsorption to the nanoparticle, as it has been demonstrated that drug-nanoparticle adsorption effect is reduced under this method in lieu of the pelleting of nanoparticles reducing the total surface area for adsorption (Zambito et al., 2012).

One of the weaknesses in this method lies in the effect of the separation step towards the data acquired in this study. Firstly, complete separation may not actually be achieved even in extreme conditions which thereby may cause higher apparent drug release compared to the actual value (Jung et al., 2018; Wallace et al., 2012; Weng et al., 2020). Secondly, it is possible for the separation step to disturb the equilibrium due to the force applied during the separation step (Jung et al., 2018; Modi & Anderson, 2013), which thereby might induce higher drug release from the nanoparticle. Thirdly, separation steps which take long period of time to complete associated with ultracentrifugation prohibit that data being assumed to reflect the instance defined as sampling time (Wallace et al., 2012). Fourthly, filtration-related separation steps, whether pressure-assisted or centrifugation-assisted, may yield a low volume of filtrate, which may not accurately represent the actual free drug concentration in the sample as the filter membrane needs to be saturated with the drug prior to effective filtration (Wallace et al., 2012). By the very nature of the method, several separation techniques can be used. The findings in current literature are summarised under **Table 2**.

Table 2: Separation methods used under the sample-and-separate method and their key consideration.

Methods	Key considerations	References
Centrifugation	• Long centrifugation time • Disrupts the equilibrium of nanoparticle-bound drugs Has inferior separation yield compared to other separation steps, especially if nanoparticles and surrounding media have insignificant density difference	(Jung et al., 2018; Modi & Anderson, 2013; Wallace et al., 2012; Weng et al., 2020)
Filtration	Requirement of higher volumes due to the adsorption of drug onto the filter membrane	(Wallace et al., 2012)
Centrifugation-assisted filtration	Requirement of higher volumes due to the adsorption of drug onto the filter membrane	(Wallace et al., 2012)

To overcome the low filtrate volume associated with the centrifugation-assisted filtration, one may increase the centrifugation speed and time of the nanoparticle to improve volume yield (Weng et al., 2020). However, such an approach should be done with consideration that the more these parameters are increased, the more chances for deformation to occur, disrupting nanoparticle integrity, and thus, the advantage of using this method, ie. a gentler condition compared to the centrifugation method would be lost (Wallace et al., 2012).

Here, a validation method *via* the usage of dynamic light scattering (DLS) or nanoparticle tracking analysis (NTA) should be considered to quantify the amount of particulate seen in the filtrate or supernatant. The count rate, expressed as kilo counts per second, would then be compared to the count rate measurement of the original media, such as drug release media, to determine whether complete separation has taken place. This is the method used in several studies to ascertain the separation of the nanoparticles from the media in many studies (Jung et al., 2018; Wallace et al., 2012; Weng et al., 2020). The filtration step, which opens the possibility of low drug concentration detected due to adsorption effect, also needs to be validated. Thus, from the same studies, the usage of drug recovery validation method should be considered, whereby free dissolved drug solutions of known concentrations are filtered through the filter membrane, and the concentration of drug in the resulting filtrate is measured *via* spectrophotometric method. The measured concentration is then compared with original concentration to quantify the drug loss resulting from the process (Jung et al., 2018; Weng et al., 2020).

It was mentioned earlier in the current review that apparent drug concentration in the drug release media may be altered due to disturbance in equilibrium during separation steps. A research strategy can be implemented to validate that this effect is not significant to the quantification of drug. This strategy involves examining how drug content, nanoparticle size of filtered nanoparticle, particles detected in filtrate using DLS method, and the volume yield changes according to the changes in either centrifugal force or time. Firstly, one of the factors of centrifugal force or time is fixed, while the other factor is varied. Test samples containing nanoparticles are then tested under these conditions, and the aforementioned parameters are measured. Next, they are then compared to determine suitability of the centrifugation procedure (Weng et al., 2020).

Direct measurement method

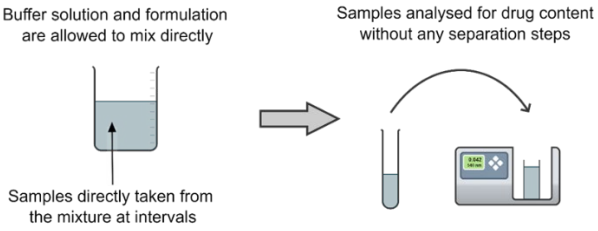

Fig. 5: The schematic diagram representing the direct measurement method of drug release studies.

Only one article was found citing the use of the direct measurement method. In this method, after sampling, the separation of nanoparticles from the sampled volume was not conducted. Instead, the concentration of the drug in the sample was analysed using UV-Vis spectrophotometry directly, using the empty nanoparticles dispersed in similar media as a blank. A schematic diagram representing the direct measurement method is shown in **Figure 5**. This accounts for the absorbance caused by the nanoparticle dispersion to be taken into account (Karimi et al., 2018). However, the usage of this technique is based on the assumption that the nanoparticle is highly reproducible in terms of size and PDI, and has low inter-batch standard deviation, as well as the assumption that loaded and empty nanoparticles have similar sizes. Here, it is erroneous to neglect proving the second assumption to be true, as it has been shown in some cases that drug-loaded and empty nanoparticles may be differ vastly in terms of size and PDI that merits ascertaining the truth of the second assumption (J. Wang et al., 2014; Yan et al., 2018). This does not take into account the possibility that changes in nanoparticle size may differ for loaded and unloaded nanoparticles. Overall, ascertaining the accuracy of this method is difficult. The usage of this approach as a potentially new method needs to be further investigated and optimised before it may be used with certain validity.

Quantification of drug release

The findings of drug release studies in the articles included are generalised here. Overall, the trends in drug release can be explained by how the ionisation of the drugs and nanoparticle constituents interact with each other. Generally, for nanoparticle complexes that encapsulate cationic drugs, more drug is released in acidic media. Meanwhile, for nanoparticle complexes that encapsulate anionic drugs, more drugs are released at higher pH. This is an observation that tends to occur under the studies included. It is possible that this is because at lower pH, protonation causes ionisation of cationic drugs and polymer, and deionisation of anionic polymer, which thus causes increased repulsion between the nanoparticle and ionic drug as well as increased passive diffusion into the hydrophilic environment. Meanwhile, the opposite is true for anionic polymer in neutral or a more basic pH. The increased repulsion force and heightened dissolution manifest themselves as increased drug release. Here, evidence that this generalisation is the most significant mechanism compared to other mechanisms is discussed.

It was found that in magnetic nanoparticles comprising of chitosan core and carrageenan outer layer, more drug is released in acidic media compared to neutral media (Karimi et al., 2018). Meanwhile, magnetic nanoparticles with carrageenan core and chitosan outer layer, despite being synthesised in the opposite manner, show the same observation (Jafari et al., 2021). Here, it should be noted that in both of these studies, sunitinib malate was the encapsulated drug, which exists as a cation at neutral pH and below (pKa = 9.8). Comparatively, chitosan and carrageenan are more protonated at acidic pH (pKa = 6.5 and pKa < 2.5 respectively), giving rise to a more charged cationic chitosan and less charged anionic carrageenan. Therefore, at lower pH, repulsive forces between the nanoparticles and sunitinib cation is more prevalent, owing up to the higher extent of cationic ionisation of the drug and chitosan, as well as the reduction of the anionic charge of carrageenan (Jafari et al., 2021; Karimi et al., 2018). Based on the two studies cited, it is possible that the configuration of which polymer constitutes the core, and which constitutes the surface is less relevant than the extent of

ionisation.

A similar case of magnetic nanoparticles made with carrageenan as the core constituent and chitosan as the outer layer further supports this point, which instead of encapsulating cationic sunitinib, encapsulates anionic methotrexate. The findings in this study are the opposite to the studies cited above, whereby more drug is released at neutral pH compared to acidic pH (Mahdavinia et al., 2017).

In a study involving cationic doxorubicin which was encapsulated by graphene oxide-carrageenan-biotin nanoparticles, it was generally found that higher release is observed in lower pH compared to neutral pH. This has been attributed to the ionisation of doxorubicin which breaks down the non-ionic bonding of the drug and graphene oxide substituent (Vinothini et al., 2019). Similar observations were found with cationic doxorubicin-loaded magnetic nanoparticles made from alkoxysilyl-modified κ-carrageenan (Nogueira et al., 2020) and doxorubicin-loaded chitosan-pectin silicon-dioxide nanoparticle (Ji et al., 2017).

Meanwhile, for anionic 5-fluorouracil-loaded chitosan-cellulose nanocomposite formulation, the opposite observation was found which is that the nanoparticle exhibited higher drug release at neutral pH rather than gastric pH (Yusefi et al., 2021). Similar observations are found in a study of chitosan nanoparticles complexed with carboxylic curdlan containing 5-fluorouracil, which was found to be released at a higher rate in neutral pH rather than in acidic conditions (Yan et al., 2018).

Meanwhile, in a study involving carrageenan core, chitosan outer layer and tripolyphosphate crosslinker microparticles loaded with α-mangostin, it was found that drug release is much higher in acidic conditions compared to more neutral conditions, which have been attributed to protonation of the particle constituents (Nguyen et al., 2022). In another study involving α-mangostin encapsulated in tripolyphosphate-linked chitosan nanoparticles, the drug is preferentially released in acidic media rather than neutral media (Herdiana et al., 2022). α-mangostin is estimated to dissociate *via* its carbonyl group, which would be basic. Thus, based on this estimation, the generalisation still holds true. It would be expected that the protonation of both chitosan and α-mangostin causes cation-cation repulsion in addition to increased solubility in the media, and hence enhanced release at acidic pH. However, one study involving this same drug found the opposite result. Studies involving nanoparticles made with chitosan as the core and carrageenan as the outer layer that encapsulates α-mangostin found that the drug is preferentially released in neutral media compared to acidic media. However, the effect of pH on drug release rates in this study was not significant (Wathoni et al., 2021).

Several studies observed the opposite trend as those postulated above. In a study involving anionic telmisartan encapsulated by magnetic chitosan nanoparticles, more drug is released in lower pH compared to higher pH (Dhavale et al., 2021). Meanwhile, in a study of polymalic acid surface-chitosan core nanoparticles loaded at the surface with cationic doxorubicin, the drug was found to be released at a higher rate in basic pH compared to lower pH (Arif et al., 2017). In order to rationalise this, the high likelihood the drug was adsorbed on the surface rather than sequestered into the nanoparticles as most other studies have cited should be considered, since it has been proposed to be the main drug loading mechanism. Here, the evidence of preferential drug release arising due to the breakdown of polymer-drug attractive interactions is apparent. In both of the studies cited, drugs are more deionised while the polymer constituents are much ionised in their preferential release conditions. Either of these occurrences may have led to the breakdown of stable drug-surface polymer interactions, hence causing enhanced release.

Another study also found the opposite observation than the generalised trend, involving PLGA-chitosan nanoparticle complexed with tripolyphosphate and coated with eucalyptus oil

for the controlled delivery of anionic 5-fluorouracil. It was found that maximum release was seen at lower pH compared to higher pH levels. Although results are not significant (Sahu et al., 2017), the effect is consistent enough that it warrants examination. Based on the findings of the research, it seems that despite the likelihood of 5-fluorouracil being sequestered inside the nanoparticle matrix, the current generalisation does not seem to apply here. However, this is an explainable exception as in this study, eucalyptus oil was used to coat the nanoparticle surface. Hence, due to hydrophobic-hydrophilic partitioning effect, deionised 5-fluouracil in acidic conditions partitioned more in the oily layer compared to ionised 5-fluouracil in basic conditions.

In the discussion, two studies are excluded from consideration as the drug release conditions are not equal in terms of other factors other than pH. One of the studies involves curcumin-loaded nanoparticles made from modified citrus pectin and chitosan meant for colon cancer, in which more drug release was observed in caecal conditions rather than gastric conditions (Sabra et al., 2018). The reason why this study could not be included to be generalised together with the other studies is that the caecal conditions in this study have enzymes, while the gastric condition does not. This opens up the possibility that the disparity in drug release was due to the enzymatic breakdown rather than the simple kinetics described above. A study involving a zein-Tween 80-carrageenan nanoparticle which encapsulates curcumin was also excluded from the current generalisation, whereby higher burst release was found in simulated intestinal fluid rather than in gastric fluid (Sun et al., 2020). This is because the presence of bile salts in the study may have been the major contributor to higher drug release in intestinal conditions. However, both of the studies cited above are similar in terms of their findings. Additionally, it was proposed that their findings were due to the lower solubility of curcumin, as well as the preference of tightly-knit formation over swollen formation at lower pH (Sabra et al., 2018). This theory is particularly supported by another study which reported opposite observation with curcumin-loaded chitosan-based nanoparticles, which might be caused by the opposite swelling response seen (Shafiee et al., 2019). Presence of bile salts and peptide in intestinal phase is also believed to further cause the difference in drug release in the two media (Sun et al., 2020).

In addition to the extent of ionisation, the swelling capacity of the nanoparticle might also play a major role, as it has been found that the more swellable non-magnetic chitosan-carrageenan nanoparticles have a higher rate of drug release compared to its swell-resistant magnetic counterpart (Mahdavinia et al., 2017). This is a theory that is supported in the findings and discussion of studies conducted by another nanoparticle as well (Arif et al., 2017; Dhavale et al., 2021; Nogueira et al., 2020; Sabra et al., 2018; Yusefi et al., 2021). However, similar mechanisms have also been used to explain reduced drug release rate (Yan et al., 2018), which thus makes the effect of nanoparticle swelling on drug release rate to remain inconclusive.

To summarise, the generalisation that drug release rate highly relies on drug-nanoparticle interaction arising due to ionisation state of its constituent is apparent. Most of the studies cited here are in agreement with this mechanism, and most of the findings in studies which disagree with the mechanism proposed are explainable by their nature of encapsulation *via* adsorption on the surface, as well as the hydrophobic nature of the nanoparticle surface. Future studies may be conducted to confirm this effect.

The following trends are also highlighted. It was found that when higher molecular weight chitosan is used as a core, less drug is released over time regardless of pH changes (Herdiana et al., 2022; Karimi et al., 2018). It was theorised that such an observation is seen due to the higher amount of amine group in high molecular weight chitosan which thereby allows a greater extent of interaction with oppositely charged constituent in the network, and hence allowing the nanoparticle to bind to the drug more tightly

(Herdiana et al., 2022). Under the same reasoning, the higher the degree of deacetylation, the lower the rate of degradation and erosion of nanoparticle surface, and thus the lower is the drug released (Sahu et al., 2017).

An initial burst release followed by sustained release was observed, whereby it was hypothesised that the burst release was due to the drug, which was released from the surface, while the sustained release was due to the drug which was released slowly over time from the matrix. The drug content also seems to heavily influence the drug release, as it was seen that nanoparticles with higher drug content have a slower release kinetics compared to nanoparticles with lower drug content (Nguyen et al., 2022).

In a study involving a complex electrospun polyvinyl alcohol/carrageenan/gold/pegylated polyurethane nanoparticle loaded with paclitaxel and camptothecin, it was found that drug release behaviour may differ according to not only the constituent of the nanoparticle, but also the physical structure. In this study, nanoparticles that were made into a composite had a burst release profile followed by a sustained release profile. However, nanoparticles that were made into a core-shell configuration had no observable burst release effect and provided a sustained release throughout the investigation (Irani & Nodeh, 2022).

Pharmacokinetically-Relevant Studies

Physicochemical response towards the environment

Some of the literature reviewed reports their investigations on the nanoparticle's response towards their environment, which are often characterised as changes in size, PDI and zeta potential. From this data, several characteristics of the nanoparticle can be inferred or proven, such as the relationship between physichochemical characteristics and the ionisation behaviour of the polymer used (Sabra et al., 2018, 2019; Shafiee et al., 2019; Sun et al., 2020; Yusefi et al., 2021), interactions with physiological constituent (Sabra et al., 2018, 2019), cancer selectivity (Yusefi et al., 2021) as well as stability under working, storage, transit and extreme conditions (Sabra et al., 2018, 2019; Sun et al., 2020; Yusefi et al., 2021). All of these characteristics may also be significantly related to drug release mechanism and may aid with understanding or explaining the observations seen in drug release studies, as demonstrated in some studies (Sabra et al., 2018; Shafiee et al., 2019; Yusefi et al., 2021). Other than that, response of the nanoparticles towards the environment may give insight on its storage stability (K. Liu et al., 2020). In some studies, the usage of physicochemical response towards presence of mucin may give insight to mucoadhesive properties of the nanoparticle (Sabra et al., 2018, 2019).

Additionally, other than the dynamic changes of the nanoparticle that has already been formed, investigations on the effect of changes in pH and salt environments of solutions during the formation of the nanoparticle towards the physicochemical characteristics of the nanoparticles have also been reported (Yan et al., 2018). These types of investigation are relevant in terms of understanding the nature in which the nanoparticle is formed, as well as the allowing the prediction of behaviour of the nanoparticle in physiological condition (Yan et al., 2018). However, applicability other than for the aim of optimisation is not clearly established. It is also possible that while this method of characterisation can prove the role of characteristics of nanoparticle constituent better than the first approach, it does lack transferability of results to the actual response that the nanoparticle will undertake when exposed to a change in environment. For the sake of simplicity, the first approach in the paragraph above is categorised as "dynamic response" while the approach stated here is categorised as "formulation response"

Generally, the test can differ in one literature over another, but they largely follow a basic premise of measuring the changes in the characteristics of the nanoparticle mentioned above after a certain condition has been changed. In both the dynamic response and formulation response, the overall procedure may involve investigating the response of the nanoparticle to conditions resembling physiological conditions in terms of pH, salt content and temperature (Sabra et al., 2018, 2019; Yusefi et al., 2021), or the effect of the three aforementioned factors are investigated one at a time (Arif et al., 2017; Sun et al., 2020; Yan et al., 2018). However, in dynamic response, the procedure can further be described to fall under two categories, which is measurement of immediate response and continuous response, the former is the measurement in only one instance (Sun et al., 2020; Yusefi et al., 2021), while the latter is the measurement of response at multiple instances at multiple time points (Sabra et al., 2018, 2019; Yusefi et al., 2021).

The procedure for formulation response is simpler to plan yet may require more resources to complete. To conduct this study, one generally has to fix other major factors affecting nanoparticle formation, while changing the conditions of one chosen factor. For example, in one case, investigations on the effect of pH on formation of polyelectrolyte complexes requires highly controlled adjustment of pH and keeping the salt content, mass content and ratio of the constituent solutions constant (Yan et al., 2018). As mentioned before, the results of this study may give insight on the nature of the nanoparticle, but does not give sufficient information that may allow accurate predictions of the nanoparticle's response towards the environment.

Meanwhile, the procedure for dynamic response may be more complex to execute but requires smaller amount of resources. The procedure largely follows the same scheme as drug release studies, i.e. selection of media pH, salt content and temperature. However, instead of measurements of drug released, nanoparticle size, PDI and zeta potential are measured instead (Ji et al., 2017; K. Liu et al., 2020; Sabra et al., 2018, 2019; Sahu et al., 2017; Shafiee et al., 2019; Sun et al., 2020; Yusefi et al., 2021). Thus, unlike in drug release studies, separation of nanoparticles is unneeded. Measurement of size, PDI and zeta potential is conducted directly upon dilution with similar media, which allows postulations regarding how the nanoparticulate system works in different environments to be better understood.

One should be cautious when interpreting the size profile of the nanoparticles, as the nanoparticle may exhibit aggregation and deposition in response to changes to the environment, which in turn would cause size changes to be underestimated (Sun et al., 2020). Here, a procedure may be considered which may help ascertain the extent of aggregation and deposition, such as the usage of DLS to measure turbidity in terms of kilo counts per second (Jung et al., 2018; Wallace et al., 2012; Weng et al., 2020), or measurement of turbidity in UV-Vis spectrophotometry to quantify the absorbance (Yan et al., 2018). However, this comes with the assumption that in these measurements, aggregated and deposited nanoparticles is not included into the sample being measured.

Mucoadhesive properties of nanoparticles may also be assessed *via* its response to essential secretion contents. In one study, mucoadhesion was assessed by measuring changes in surface charge/zeta potential after allowing it to interact with mucin at predetermined time. The changes in zeta potential were measured in different mucin concentrations, at different pH; pH 1.2 to simulate gastric conditions and pH 7.0 to simulate colon conditions. A drop in zeta potential due to mucin interactions was

explained by the electrostatic interactions between nanoparticle surface and mucin. Due to this method, the study have successfully proven selective mucoadhesive properties of the nanoparticle to the colon mucosa rather than gastric mucosa (Sabra et al., 2019). Alternatively, mucoadhesion studies were also conducted by allowing the nanoparticles to mix with mucin and centrifuging the nanoparticles. The supernatant was then mixed with micro-BSA and was left to incubate followed by measurement of absorbance at 562 nm. In principle, this allows the measurement of free mucin which thereby allows the quantification of the extent of mucin-nanoparticle adhesion. In this variation of study on the same nanoparticles, similar observation was found (Sabra et al., 2018).

Here, the findings reported in the literature included in the review are generalised. Under dynamic response, generally, nanoparticles made from chitosan swells more at lower pH compared to higher pH (Ji et al., 2017; C. Liu et al., 2020; Sahu et al., 2017; Shafiee et al., 2019). However, some studies reported higher extent of swelling in higher pH conditions (Sabra et al., 2018; Yusefi et al., 2021). So far, there has not been a clear reasoning on why the studies reported contradictory findings. However, in general, the swelling effect is even greater where proteins with opposite charge to the surface of the nanoparticle are present in the media (Sabra et al., 2019). The extent of this swelling can be reduced by complexation with modified citrus pectin (Sabra et al., 2018), or increased by complexation with cellulose fibre (Yusefi et al., 2021).

Meanwhile, for formulation response, changes in physicochemical properties are attributable to the extent of ionisation of the nanoparticle constituent. In a study of carboxylic curdlan and chitosan nanoparticle, if the polymer solutions' pH prior to complexation is increased from pH 3.0 to 5.0, the size of the nanoparticle increases. This is believed to be due to the deprotonation of amine groups of chitosan, which decreases the extent of ionic attraction between chitosan and the anionic polymer. However, there has also been an observation whereby the chitosan-based nanoparticles may swell if it is too acidic, which is associated with the change in extent of ionisation of non-chitosan substituent which thereby decreases the extent of ionic interaction between chitosan and the ionisable anionic substituent (Yan et al., 2018). A relatively similar observation was found in a polymalic acid-chitosan nanoparticle. It was found that the nanoparticles remain in relatively similar sizes at pH 1.2-6.0 range, while a significant increase is observed at pH 7.4. This study also explains this observation as attributable to the deprotonation of chitosan which reduces the extent of complexation. However, this result is counterintuitive to the fact that the pKa of chitosan which is close to 6.5, while polymalonic acid having a pKa of 3.4-3.6. It was proposed here that instead of ionic complexation, the polymalonic acid and chitosan interact and entangle one another *via* electrostatic attractions (Arif et al., 2017).

As studies involving nanoparticle's response to environmental conditions are rather scarce for carrageenan-based nanoparticles under the pre-defined scope, a generalisable statement could not be drawn regarding their response to stimuli.

Simulated digestion

In one study involving zein-chitosan nanoparticle loaded with curcumin, the effect of digestion on size, morphology and curcumin bioaccessibility were assessed, whereby the nanoparticles are undergoing simulated digestion, which is incubation and agitation in simulated gastric fluid for 2 hours followed by simulated intestinal fluid for 2 hours, at 37°C. The mixtures are then centrifuged at 10°C and 10,000 rpm for 40 minutes, followed by filtration of the

supernatant with 0.45μm membrane filter before analysis for curcumin content. It was found that the size of the nanoparticles increased greatly in gastric conditions over time, while no changes were found in intestinal conditions. It was explained that morphological changes which occur could be due to the effect of pancreatin, pepsin and acidic or basic environments which act on the surface of the nanoparticles. Interestingly, the results of curcumin bioaccessibility indicated that free curcumin and curcumin loaded into chitosan nanoparticles is not stable in digestive conditions, but the curcumin loaded into zein nanoparticles were more stable, followed by chitosan-zein nanoparticles. This indicates that chitosan works synergistically with zein to protect curcumin from degradation (C. Liu et al., 2020).

Storage Stability Studies

Method of characterisation of stability

The storage stability studies are the least conducted study between all of the reviewed studies. Thus, due to this, reference for generalisation under this study is scarce. In the current review, it was found that the following parameters outlined in Table 3 were measured for considerations of stability.

Table 3: The parameters measured as an indication of stability in storage conditions.

Parameters	Reference
General appearance (Turbidity, deposition and/or colour)	(Gaur et al., 2022; K. Liu et al., 2020; Sun et al., 2020)
Nanoparticle size and PDI	(Gaur et al., 2022; C. Liu et al., 2020; K. Liu et al., 2020; Sabra et al., 2019; Sun et al., 2020)
Zeta potential	(Gaur et al., 2022; K. Liu et al., 2020; Sun et al., 2020)
Morphological changes	(Sabra et al., 2019)
Encapsulation efficiency	(Gaur et al., 2022; Sabra et al., 2019)
Drug loading capacity / drug content	(Gaur et al., 2022; C. Liu et al., 2020; Sabra et al., 2019)
Drug release studies	(Gaur et al., 2022)
Ease of reconstitution	(Gaur et al., 2022)
Enzyme activity	(Moradi et al., 2021)

The stability studies of all of the chitosan and/or carrageenan-based nanoparticles reported in this review were conducted either on one formulation which allows definitive interpretation to be made (Gaur et al., 2022; K. Liu et al., 2020; Sabra et al., 2019), or on multiple formulation which also allows comparative discussion (C. Liu et al., 2020; K. Liu et al., 2020; Moradi et al., 2021; Sun et al., 2020).

Generally, the length of study and measurement time points greatly differ from study to study. However, it can be classified that most stability studies reported were planned to take at least one month to be completed (a period of 30 days), while in between, regular 5 day or weekly intervals of testing were conducted. In some cases, changes in parameters are only assessed at the end of the period (C. Liu et al., 2020; K. Liu et al., 2020). In the case whereby significant changes in the parameter occur, it can be concluded that the nanoparticle is no longer stable, the stability study may be terminated in the case where only one formulation is tested (Sabra et al., 2019), or it may be prolonged to allow comparative analysis between different formulations (Moradi et al., 2021; Sun et al., 2020).

In all of the studies cited, temperature remains the focus as the factor that is controlled for the stability studies. In all studies, there are no mention of pre-defined humidity levels used to conduct the study. It should be noted that humidity effect is an important parameter to be considered as the stability of encapsulated drug may be highly affected by the addition or removal of water from the crystalline structure of drug; in some

cases, drugs may take anhydrous forms which are more unstable (Santamaría-Aguirre et al., 2018). Polymeric degradation under different moisture conditions are considerable, as changes in relative humidity leads to changes in water content of the polymer, which has been shown to affect chitosan and carrageenan stability and degradation (Friedenthal et al., 2020; Shahbazi et al., 2016). As such, where available and possible, stability studies should report on the humidity on top of temperature as it is an important consideration on stability as well.

It can be said that protection of the active pharmaceutical ingredient (Moradi et al., 2021) and increased stability of the nanoparticle (C. Liu et al., 2020) can be seen with the usage of chitosan polymer in nanoparticle formulation. A mid-range polymer concentration showed the greatest stability in a study involving carrageenan-based nanoparticles (Sun et al., 2020).

Size changes are associated with Ostwald ripening, whereby small size of the nanoparticles causes favoured deposition on bigger particles due to higher surface area to volume ratio. Additionally, drug loading capacity and encapsulation efficiency decreased, which was explained by the autocatalytic reaction of bigger nanoparticles thereby leading to release of drugs (Sabra et al., 2019).

Conclusion

Studies involving in vitro kinetics of nanoparticle characteristics have been widespread in nanoparticle research for the objective of understanding the behaviour of nanoparticles in its application. However, due to lack of standardisation in the procedures conducted owing up to the differences in application and nanoparticle properties, differences between study results arise which makes it highly subject to inaccuracies arising due to unsuitable or incomplete study methods. The current literature review generalises the trends in the methods used in drug release profiling, pharmacokinetically-relevant studies and storage stability studies under the context of chitosan and/or carrageenan nanoparticles in anticancer application. The current review also highlights several areas which may be improved to increase accuracy in the studies conducted, which may be adapted to improve analysis in future studies. With these generalisations and suggestions, the current literature review will help researchers to be able to plan their future studies to be not only more accurate, but also potentially more comparable and similar in terms of methodology with other studies. Next up, the results of the drug release studies may also be generalisable and are explainable by the physical and chemical characteristics of constituent, particularly with the response of drug release towards changes in pH which are explainable *via* the principles of ionisation. Hence, there is a possibility that in the future, hypotheses or predictions in their kinetic behaviours can be made. In the future, these trends should be examined further under a more broadened scope to conclusively determine whether they still hold true in polymeric nanoparticles made from other materials.

Authors contributions

Conception and design of the work, A.U.; acquisition, analysis, and interpretation of articles, A.U.; writing—original draft preparation, A.U.; writing—editing, A.U.; writing—review, I.F.M.S., H.H., S.F.C.O.; writing—design and formatting, A.U., I.F.M.S.; visualization, A.U.; supervision, I.F.M.S., H.H., S.F.C.O.; project administration, I.F.M.S. All authors have read and agreed to the published version of the manuscript.

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References

Abouelmagd, S. A., Sun, B., Chang, A. C., Ku, Y. J., & Yeo, Y. (2015). Release kinetics study of poorly water-soluble drugs from nanoparticles: Are we doing it right? *Molecular Pharmaceutics*, *12*(3), 997–1003. https://doi.org/10.1021/mp500817h

Ali, A., & Ahmed, S. (2018). A review on chitosan and its nanocomposites in drug delivery. *International Journal of Biological Macromolecules*, *109*, 273–286. https://doi.org/10.1016/j.ijbiomac.2017.12.078

Al-Samydai, A., Al-Mamoori, F., Abdelnabi, H., & Aburjai, T. (2019). An updated review on anticancer activity of capsaicin. *International Journal of Scientific and Technology Research*, *8*(12), 2625–2630.

Arif, M., Raja, M. A., Zeenat, S., Chi, Z., & Liu, C. (2017). Preparation and characterization of polyelectrolyte complex nanoparticles based on poly (malic acid), chitosan. A pH-dependent delivery system. *Journal of Biomaterials Science, Polymer Edition*, *28*(1), 50–62. https://doi.org/10.1080/09205063.2016.1242460

Bourbon, A. I., Pinheiro, A. C., Cerqueira, M. A., & Vicente, A. A. (2018). In vitro digestion of lactoferrin-glycomacropeptide nanohydrogels incorporating bioactive compounds: Effect of a chitosan coating. *Food Hydrocolloids*, *84*, 267–275. https://doi.org/10.1016/j.foodhyd.2018.06.015

Chu, X., Huang, W., Wang, Y., Meng, L., Chen, L., Jin, M., Chen, L., Gao, C., Ge, C., Gao, Z., & Gao, C. (2019). Improving antitumor outcomes for palliative intratumoral injection therapy through lecithin–chitosan nanoparticles loading paclitaxel–cholesterol complex. *International Journal of Nanomedicine*, *14*, 689–705. https://doi.org/10.2147/IJN.S188667

Dhavale, R. P., Dhavale, R. P., Sahoo, S. C., Kollu, P., Jadhav, S. U., Patil, P. S., Dongale, T. D., Chougale, A. D., & Patil, P. B. (2021). Chitosan coated magnetic nanoparticles as carriers of anticancer drug Telmisartan: pH-responsive controlled drug release and cytotoxicity studies. *Journal of Physics and Chemistry of Solids*, *148*. https://doi.org/10.1016/j.jpcs.2020.109749

Fan, L., Duan, M., Sun, X., Wang, H., & Liu, J. (2020). Injectable Liquid Metal- And Methotrexate-Loaded Microsphere for Cancer Chemophotothermal Synergistic Therapy. *ACS Applied Bio Materials*, *3*(6), 3553–3559. https://doi.org/10.1021/acsabm.0c00171

Friedenthal, M., Eha, K., Kaleda, A., Part, N., & Laos, K. (2020). Instability of low-moisture carrageenans as affected by water vapor sorption at moderate storage temperatures. *SN Applied*

Sciences, *2*(2). https://doi.org/10.1007/s42452-020-2032-9

Gaur, P. K., Puri, D., Singh, A. P., Kumar, N., & Rastogi, S. (2022). Optimization and Pharmacokinetic Study of Boswellic Acid–Loaded Chitosan-Guggul Gum Nanoparticles Using Box-Behnken Experimental Design. *Journal of Pharmaceutical Innovation*, *17*(2), 485–500. https://doi.org/10.1007/s12247-020-09527-0

Herdiana, Y., Wathoni, N., Shamsuddin, S., & Muchtaridi, M. (2022). Cytotoxicity Enhancement in MCF-7 Breast Cancer Cells with Depolymerized Chitosan Delivery of α-Mangostin. *Polymers*, *14*(15). https://doi.org/10.3390/polym14153139

Hoshyar, N., Gray, S., Han, H., & Bao, G. (2016). The effect of nanoparticle size on in vivo pharmacokinetics and cellular interaction. *Nanomedicine*, *11*(6), 673–692. https://doi.org/10.2217/nnm.16.5

Hotchkiss, S., Brooks, M., Campbell, R., Philp, K., & Trius, A. (2016). The Use of Carrageenan in Food. In L. Pereira (Ed.), *Carrageenans - Sources and Extraction Methods, Molecular Structure, Bioactive Properties and Health Effects* (1st ed., pp. 229–243). Nova Science Publishers.

Irani, M., & Nodeh, S. M. (2022). PVA/κ-carrageenan/Au/camptothecin/pegylated-polyurethane/paclitaxel nanofibers against lung cancer treatment. *RSC Advances*, *12*(25), 16310–16318. https://doi.org/10.1039/d2ra02150a

Jafari, H., Atlasi, Z., Mahdavinia, G. R., Hadifar, S., & Sabzi, M. (2021). Magnetic κ-carrageenan/chitosan/montmorillonite nanocomposite hydrogels with controlled sunitinib release. *Materials Science and Engineering C*, *124*. https://doi.org/10.1016/j.msec.2021.112042

Jennings, J. A. (2017). Controlling chitosan degradation properties in vitro and in vivo. In *Chitosan Based Biomaterials* (Vol. 1, pp. 159–182). Elsevier Inc. https://doi.org/10.1016/B978-0-08-100230-8.00007-8

Ji, F., Li, J., Qin, Z., Yang, B., Zhang, E., Dong, D., Wang, J., Wen, Y., Tian, L., & Yao, F. (2017). Engineering pectin-based hollow nanocapsules for delivery of anticancer drug. *Carbohydrate Polymers*, *177*, 86–96. https://doi.org/10.1016/j.carbpol.2017.08.107

Jung, F., Nothnagel, L., Gao, F., Thurn, M., Vogel, V., & Wacker, M. G. (2018). A comparison of two biorelevant in vitro drug release methods for nanotherapeutics based on advanced physiologically-based pharmacokinetic modelling. *European Journal of Pharmaceutics and Biopharmaceutics*, *127*, 462–470. https://doi.org/10.1016/j.ejpb.2018.03.010

Karimi, M. H., Mahdavinia, G. R., & Massoumi, B. (2018). pH-controlled sunitinib anticancer release from magnetic chitosan nanoparticles crosslinked with κ-carrageenan. *Materials Science and Engineering C*, *91*, 705–714. https://doi.org/10.1016/j.msec.2018.06.019

Kharkwal, H., & Janaswamy, S. (Eds.). (2017). *Natural Polymers for Drug Delivery*. CAB International.

Liu, C., Yuan, Y., Ma, M., Zhang, S., Wang, S., Li, H., Xu, Y., & Wang, D. (2020). Self-assembled composite nanoparticles based on zein as delivery vehicles of curcumin: Role of chondroitin sulfate. *Food and Function*, *11*(6), 5377–5388. https://doi.org/10.1039/d0fo00964d

Liu, K., Huang, R. L., Zha, X. Q., Li, Q. M., Pan, L. H., & Luo, J. P. (2020). Encapsulation and sustained release of curcumin by a composite hydrogel of lotus root amylopectin and chitosan. *Carbohydrate Polymers*, *232*. https://doi.org/10.1016/j.carbpol.2019.115810

Mahdavinia, G. R., Mosallanezhad, A., Soleymani, M., & Sabzi, M. (2017). Magnetic- and pH-responsive κ-carrageenan/chitosan complexes for controlled release of methotrexate anticancer drug. *International Journal of Biological Macromolecules*, *97*, 209–217. https://doi.org/10.1016/j.ijbiomac.2017.01.012

Modi, S., & Anderson, B. D. (2013). Determination of drug release kinetics from nanoparticles: Overcoming pitfalls of the dynamic dialysis method. *Molecular Pharmaceutics*, *10*(8), 3076–3089. https://doi.org/10.1021/mp400154a

Moradi, R., Mohammadzadeh, R., & Akbari, A. (2021). Kappa-Carrageenan Crosslinked Magnetic Folic Acid-Conjugated Chitosan Nanocomposites for Arginase Encapsulation, Delivery and Cancer Therapy. *Nano LIFE*, *11*(03), 2140005. https://doi.org/10.1142/S1793984421400055

Moreno-Bautista, G., & Tam, K. C. (2011). Evaluation of dialysis membrane process for quantifying the in vitro drug-release from colloidal drug carriers. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *389*(1–3), 299–303. https://doi.org/10.1016/j.colsurfa.2011.07.032

Mulet-Cabero, A. I., Egger, L., Portmann, R., Ménard, O., Marze, S., Minekus, M., Le Feunteun, S., Sarkar, A., Grundy, M. M. L., Carrière, F., Golding, M., Dupont, D., Recio, I., Brodkorb, A., & Mackie, A. (2020). A standardised semi-dynamic: in vitro digestion method suitable for food-an international consensus. *Food and Function*, *11*(2), 1702–1720. https://doi.org/10.1039/c9fo01293a

Narayanaswamy, R., & Torchilin, V. P. (2019). Hydrogels and Their Applications in Targeted Drug Delivery. *Molecules*, *24*(3), 603. https://doi.org/10.3390/molecules24030603

Nguyen, T. H., Nguyen, T. C., Nguyen, T. M. T., Hoang, D. H., Tran, D. M. T., Tran, D. T., Hoang, P. T., Le, V. T., Tran, T. K. N., & Thai, H. (2022). Characteristics and Bioactivities of Carrageenan/Chitosan Microparticles Loading α-Mangostin. *Journal of Polymers and the Environment*, *30*(2), 631–643. https://doi.org/10.1007/s10924-021-02230-2

Nogueira, J., Soares, S. F., Amorim, C. O., Amaral, J. S., Silva, C., Martel, F., Trindade, T., & Daniel-Da-Silva, A. L. (2020). Magnetic driven nanocarriers for pH-responsive doxorubicin release in cancer therapy. *Molecules*, *25*(2). https://doi.org/10.3390/molecules25020333

Rostami, E. (2020). Progresses in targeted drug delivery systems using chitosan nanoparticles in cancer therapy: A mini-

review. *Journal of Drug Delivery Science and Technology*, *58*, 101813. https://doi.org/10.1016/j.jddst.2020.101813

Sabra, R., Billa, N., & Roberts, C. J. (2018). An augmented delivery of the anticancer agent, curcumin, to the colon. *Reactive and Functional Polymers*, *123*, 54–60. https://doi.org/10.1016/j.reactfunctpolym.2017.12.012

Sabra, R., Roberts, C. J., & Billa, N. (2019). Courier properties of modified citrus pectinate-chitosan nanoparticles in colon delivery of curcumin. *Colloid and Interface Science Communications*, *32*, 100192. https://doi.org/10.1016/j.colcom.2019.100192

Sahatsapan, N., Rojanarata, T., Ngawhirunpat, T., Opanasopit, P., & Patrojanasophon, P. (2021). Doxorubicin-loaded chitosan-alginate nanoparticles with dual mucoadhesive functionalities for intravesical chemotherapy. *Journal of Drug Delivery Science and Technology*, *63*. https://doi.org/10.1016/j.jddst.2021.102481

Sahu, P., Kashaw, S. K., Jain, S., Sau, S., & Iyer, A. K. (2017). Assessment of penetration potential of pH responsive double walled biodegradable nanogels coated with eucalyptus oil for the controlled delivery of 5-fluorouracil: In vitro and ex vivo studies. *Journal of Controlled Release*, *253*, 122–136. https://doi.org/10.1016/j.jconrel.2017.03.023

Santamaría-Aguirre, J., Alcocer-Vallejo, R., & López-Fanárraga, M. (2018). Drug Nanoparticle Stability Assessment Using Isothermal and Nonisothermal Approaches. In *Journal of Nanomaterials* (Vol. 2018). Hindawi Limited. https://doi.org/10.1155/2018/3047178

Shafiee, S., Ahangar, H. A., & Saffar, A. (2019). Taguchi method optimization for synthesis of Fe3O4 @chitosan/Tragacanth Gum nanocomposite as a drug delivery system. *Carbohydrate Polymers*, *222*. https://doi.org/10.1016/j.carbpol.2019.114982

Shahbazi, M., Rajabzadeh, G., Ettelaie, R., & Rafe, A. (2016). Kinetic study of κ-carrageenan degradation and its impact on mechanical and structural properties of chitosan/κ-carrageenan film. *Carbohydrate Polymers*, *142*, 167–176. https://doi.org/10.1016/j.carbpol.2016.01.037

Sun, X., Pan, C., Ying, Z., Yu, D., Duan, X., Huang, F., Ling, J., & Ouyang, X. kun. (2020). Stabilization of zein nanoparticles with k-carrageenan and tween 80 for encapsulation of curcumin. *International Journal of Biological Macromolecules*, *146*, 549–559. https://doi.org/10.1016/j.ijbiomac.2020.01.053

Tharkar, P., Varanasi, R., Wong, W. S. F., Jin, C. T., & Chrzanowski, W. (2019). Nano-Enhanced Drug Delivery and Therapeutic Ultrasound for Cancer Treatment and Beyond. In *Frontiers in Bioengineering and Biotechnology* (Vol. 7). Frontiers Media S.A. https://doi.org/10.3389/fbioe.2019.00324

Trousil, J., Pavliš, O., Kubíčková, P., Škorič, M., Marešová, V., Pavlova, E., Knudsen, K. D., Dai, Y.-S., Zimmerman, M., Dartois, V., Fang, J.-Y., & Hrubý, M. (2020). Antitubercular nanocarrier monotherapy: Study of In Vivo efficacy and pharmacokinetics for rifampicin. *Journal of Controlled Release*, *321*, 312–

323. https://doi.org/10.1016/j.jconrel.2020.02.026

Utreja, P., Verma, S., Rahman, M., & Kumar, L. (2020). Use of Nanoparticles in Medicine. *Current Biochemical Engineering*, *6*(1), 7–24. https://doi.org/10.2174/2212711906666190724145101

Vinothini, K., Rajendran, N. K., Munusamy, M. A., Alarfaj, A. A., & Rajan, M. (2019). Development of biotin molecule targeted cancer cell drug delivery of doxorubicin loaded κ-carrageenan grafted graphene oxide nanocarrier. *Materials Science and Engineering C*, *100*, 676–687. https://doi.org/10.1016/j.msec.2019.03.011

Wallace, S. J., Li, J., Nation, R. L., & Boyd, B. J. (2012). Drug release from nanomedicines: Selection of appropriate encapsulation and release methodology. *Drug Delivery and Translational Research*, *2*(4), 284–292. https://doi.org/10.1007/s13346-012-0064-4

Wang, J., Ni, C., Zhang, Y., Zhang, M., Li, W., Yao, B., & Zhang, L. (2014). Preparation and pH controlled release of polyelectrolyte complex of poly(l-malic acid-co-d,l-lactic acid) and chitosan. *Colloids and Surfaces B: Biointerfaces*, *115*, 275–279. https://doi.org/10.1016/j.colsurfb.2013.12.018

Wang, S., Pi, L., Wen, H., Yu, H., & Yang, X. (2020). Evaluation of novel magnetic targeting microspheres loading adriamycin based on carboxymethyl chitosan. *Journal of Drug Delivery Science and Technology*, *55*, 101388. https://doi.org/10.1016/j.jddst.2019.101388

Wathoni, N., Meylina, L., Rusdin, A., Abdelwahab Mohammed, A. F., Tirtamie, D., Herdiana, Y., Motoyama, K., Panatarani, C., Joni, I. M., Lesmana, R., & Muchtaridi, M. (2021). The potential cytotoxic activity enhancement of α-mangostin in chitosan-kappa carrageenan-loaded nanoparticle against mcf-7 cell line. *Polymers*, *13*(11). https://doi.org/10.3390/polym13111681

Weng, J., Tong, H. H. Y., & Chow, S. F. (2020). In vitro release study of the polymeric drug nanoparticles: Development and validation of a novel method. *Pharmaceutics*, *12*(8), 1–18. https://doi.org/10.3390/pharmaceutics12080732

World Health Organization. (2012). *Cancer - Key Statistics*. https://www.who.int/cancer/resources/keyfacts/en/

World Health Organization. (2020). *Malaysia - Cancer Country Profile 2020*. https://www.who.int/cancer/country-profiles/MYS_2020.pdf

Yan, J. K., Qiu, W. Y., Wang, Y. Y., Wu, L. X., & Cheung, P. C. K. (2018). Formation and characterization of polyelectrolyte complex synthesized by chitosan and carboxylic curdlan for 5-fluorouracil delivery. *International Journal of Biological Macromolecules*, *107*(PartA), 397–405. https://doi.org/10.1016/j.ijbiomac.2017.09.004

Yu, M., Yuan, W., Li, D., Schwendeman, A., & Schwendeman, S. P. (2019). Predicting drug release kinetics from nanocarriers inside dialysis bags. *Journal of Controlled Release*, *315*, 23–30.

https://doi.org/10.1016/j.jconrel.2019.09.016

Yusefi, M., Chan, H. Y., Teow, S. Y., Kia, P., Lee-Kiun Soon, M., Sidik, N. A. B. C., & Shameli, K. (2021). 5-fluorouracil encapsulated chitosan-cellulose fiber bionanocomposites: Synthesis, characterization and in vitro analysis towards colorectal cancer cells. *Nanomaterials*, *11*(7). https://doi.org/10.3390/nano11071691

Zambito, Y., Pedreschi, E., & di Colo, G. (2012). Is dialysis a reliable method for studying drug release from nanoparticulate systems? - A case study. *International Journal of Pharmaceutics*, *434*(1–2), 28–34. https://doi.org/10.1016/j.ijpharm.2012.05.020

Zhang, E., Xing, R., Liu, S., Li, K., Qin, Y., Yu, H., & Li, P. (2019). Vascular targeted chitosan-derived nanoparticles as docetaxel carriers for gastric cancer therapy. *International Journal of Biological Macromolecules*, *126*, 662–672. https://doi.org/10.1016/j.ijbiomac.2018.12.262

Zia, K. M., Tabasum, S., Nasif, M., Sultan, N., Aslam, N., Noreen, A., & Zuber, M. (2017). A review on synthesis, properties and applications of natural polymer based carrageenan blends and composites. *International Journal of Biological Macromolecules*, *96*, 282–301. https://doi.org/10.1016/j.ijbiomac.2016.11.095

Zielińska, A., Carreiró, F., Oliveira, A. M., Neves, A., Pires, B., Venkatesh, D. N., Durazzo, A., Lucarini, M., Eder, P., Silva, A. M., Santini, A., & Souto, E. B. (2020). Polymeric Nanoparticles: Production, Characterization, Toxicology and Ecotoxicology. *Molecules*, *25*(16), 3731. https://doi.org/10.3390/molecules25163731

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