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ORIGINAL ARTICLE

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Experiential education as a strategy to preserve *Maqasid Al-Shariah* by identifying and addressing stigmatic views held by pharmacy students of patients with substance use disorders.

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ABSTRACT

Introduction: Stigmatising people with a history of substance use disorders (PHSUDs) is discriminatory, causes harm by hindering access to health services and promotes relapse. It jeopardises the preservation of *Maqasid Al-Shariah*, particularly the protection of life and well-being. Experiential education (EE) is a potential strategy to overcome stigma through direct experience with PHSUDs. This study aimed to identify stigmatised views of PHSUD's held by pharmacy students and, in accord with *Maqasid Al-Shariah*, the effectiveness of EE in changing those views.

Materials and methods: Interviews were conducted with seven undergraduate pharmacy students who provided care to PHSUDs through an EE programme. A semi-structured interview guide was used to explore students' perceptions on stigma. Each interview was audio-taped, transcribed verbatim and translated into English. Data were manually sorted and coded using Microsoft Excel 2016 and subjected to thematic analysis.

Results: The following themes related to stigma were identified: 1) individuals are to blame 2) moral versus biological views of addiction 3) stereotypes of unpredictability and dangerousness 4) lack of didactic education/training for pharmacy students regarding PHSUDs and 5) lack of face-to-face experiential education with PHSUDs. Less stigma and greater empathy towards PHSUDs were reported post-intervention attributable to increased knowledge about substance abuse and face-to-face interactions with PHSUDs.

Conclusion: Pharmacy students have pre-conceived stigmatic views regarding PHSUDs modifiable through experiential education. Contact by pharmacy students with stigmatised patients promotes health care without discrimination as advocated in the teaching of Islam. Future interventions to reduce stigma are required to preserve *Maqasid Al-Shariah*.

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Introduction

Stigma complex is defined as "the set of interrelated, heterogeneous system structures, from the individual to the society, and processes, from the molecular to the geographic and historical, that constructs, labels, and translates difference into marks" (Pescosolido & Martin, 2015). Negative marks associated with substance use disorders (SUDs) include health, social and behavioural dispositions at odds with societal expectations (e.g. unemployment and crime) that result in social rejection (Committee on the Science of Changing Behavioral Health Social Norms et al., 2016). Manifestations of stigma affect health outcomes negatively. They hinder health-seeking behaviour, limit allocation of resources for treatment/intervention and dissuade health care professionals from providing services (Yang et al., 2017). Accordingly, it is important to identify and address negative views held by current and future healthcare professionals towards people with a history of substance use disorders (PHSUDs). That is especially important for Muslim healthcare professionals because holding stigmatised views is inconsistent with the Islamic principles of *Maqasid Al-Shariah* (Raysuni, 2005).

Maqasid Al-Shariah refers to the higher objectives of the Islamic law that emphasise the protection/preservation of basic human rights including five fundamental elements: faith/religion, life, lineage/offspring, intellect and wealth/property (Raysuni, 2005). Preservation of the elements can be achieved through efforts related to establishing and strengthening them and by removing potential threats such as poor health (Ibn Ashur, 2006). Stigma towards PHSUDs has been associated with poor health outcomes (Crapanzano et al., 2018).

Didactic and experiential education are effective strategies in changing stigmatised attitudes towards SUDs (Committee on the Science of Changing Behavioral Health Social Norms et al., 2016). Experiential education in pharmacy education involves direct interaction between students and patients in clinical and public healthcare settings and is an important strategy to improve the competency of future pharmacists (Legal, 2019). However, research is lacking in addressing stigma associated with SUDs among pharmacy students. This study sought 1) to identify stigmatised views held by pharmacy students towards PHSUDs and the influence of experiential education on those views and 2) to discuss the value to students of providing healthcare for PHSUDs with respect to the Islamic principles of *Maqasid Al-Shariah*.

Methodology

This study received ethical approval from the International Islamic University Malaysia (IIUM) Research Ethics Committee (ID No. IREC 2019-026). Eligibility criteria for participants were students who completed the IIUM Drug Abuse course PHM 3282, volunteered to be trained in providing face-to-face health screening services to PHSUDs and consented to be interviewed. PHSUDs were people who completed drug rehabilitation treatment and were monitored by the Malaysian government's National Anti-Drug Agency (NADA) in Kuantan, Malaysia. The activities undertaken by the students during the programme were screening of blood pressure, glucose, lipid and carbon monoxide levels.

A qualitative study that involved semi-structured face-to-face interviews was conducted with all (7) students who met the eligibility requirements, at the Faculty of Pharmacy, IIUM. It was conducted from January until May 2019 at mutually convenient times arranged by the interviewer and each student. Students were contacted by the researchers who explained the purpose and nature of the study. One-on-one interviews were conducted in the Malay language by one interviewer using a general interview guide designed by the researchers to allow students to comment on their didactic knowledge, perceptions, experience, patient interactions and reflections pertinent to the study. Questions addressing barriers, problems, challenges during the experiential learning as well as positive and negative feelings specific to PHSUDs were emphasised to obtain detailed elaboration on stigma, importance of health services to PHSUDs from educational, health and Islamic perspectives. When deemed necessary, questions were repeated and/or rephrased to confirm the students' understanding of the questions asked and to confirm the interviewer's understanding of the students' answers. The interviews were audiotaped, transcribed verbatim and translated into English. The names of participants were kept anonymous for privacy protection.

A thematic analysis of the transcripts was undertaken to identify, analyse, and report recurring themes relevant to the study (Braun & Clarke, 2006). Data (relevant phrases, sentences, etc.) were copied from each transcript to a Microsoft Excel spreadsheet and manually sorted to facilitate the identification and coding of recurring themes. That process was conducted independently by two researchers and differences in the identification and coding of themes were resolved through discussion. There is no established ideal sample size when using thematic analysis. Most qualitative studies use the concept of saturation, the point at which no new information or themes are observed in the data (Guest, Bunce & Johnson, 2006; Willig, 2013; Fugard & Potss, 2015).

Table 1: Views held by pharmacy students towards SUDs and the influence of experiential education on those views.

Themes	Quotes
Individuals are to blame	"We tend to blame the drug addicts that they don't want to try to go back to the right path…after they go for rehab, they relapse" (P6) "I had this stigma on why we should spend a large amount of money in helping them because it is their own problem. Why did they involve in drug abuse and difficult to be cured?" (P2) "mostly come from low education status... rural area, they involved with drugs influence by their friends" (P4) "drug abusers as those who are from disorganised family institution" (P5) "I felt uncomfortable because people who involved with drugs may have HIV...they were also not really friendly" (P7)
Moral versus biological views of addiction	"I learned how drugs affect the health of drug users, addiction can affect their mental health, physiological, quality of life and attitude…I can see that it is a disease, not only because of themselves" (P2) "I thought that people who took drugs just wanted to have fun, they didn't want to quit drugs…we know that withdrawal syndromes involve physiology of their body…same with other chronic diseases…so it is partly not their will" (P7)
Stereotypes of unpredictability and dangerousness	"At first I was quite scared because may be some of them could become aggressive...cheating…we don't know if they have withdrawal symptoms" (P1) "From afar, I saw that they were quite rough in terms of personality, so I was a bit scared to have a chat with them" (P4) "Concern about their behaviour...those who have difficulty to cooperate" (P5) "How do we communicate with these people...because drug (addiction) is a sensitive issue" (P6)
Lack of education/training among students	"We only learned theory previously, we know better now from experience" (P1) "Experiential learning is important. When we understand about how drugs affect them in reality…it can change our thought on them" (P2). "Those who did not take the course (IIUM Drug Abuse course PHM 3282), they lack preparation…we need to have more hands-on experiences so that students are confident to do it...no more fear of doing such activities. We can also learn from psychological aspect...or behavioural knowledge…about the best method to approach them" (P7)

Themes	Quotes
Lack of contact with PHSUDs	"There should be more exposure to ex-drug addicts in terms of teaching and learning" (P3) "it was a totally new experience, I got to know their behaviour...some of my friends did not want to involve when they knew that this program involved ex-drug users" (P4) "He told us about how many cigarettes he smoked before...this population is a bit unique...we need to know their behaviour...because different people require different methods" (P5) "This programme was an opportunity for me to meet and observe them...positive experience... I could interact with them and they shared their stories how they got involved with drugs" (P6)
Reduced stigma and increased empathy following direct contact	"Before the programme, I felt a bit scared and nervous, and some negative thought. After that I felt like they were similar to other patients...they told stories...we got some insights how hard it was for them to quit drugs…when they went to the clinic to seek treatment, the public had sceptical opinion towards them…sometimes even the hospital staff were being sarcastic and teasing them…they felt inferior, they did not want to go anymore" (P1) "It changed my stigma about them...I wanted to help them" (P2) "They said that they did not usually do health screening…so when we do it, they seemed to appreciate the services…we should get to know their community better" (P3) "In Islam, smoking cigarette is forbidden...I observed the bad things that happened to the patients from smoking based on their stories...so it strengthens the fact that smoking should be avoided completely...if we do not care about them properly they could relapse" (P5) "Improved my perspective on drug addict, not all of them wanted to involve with drugs...a patient told us that he took morphine to relieve the pain due to an illness...then he got addicted" (P6) "this programme gave us more understanding and empathy towards this population" (P7)

Saturation was deemed to have occurred in this study after five students were interviewed. Data triangulation process related to stigma and substance use disorders, as well as Islamic concept of both components in relation to *Maqasid al-Shariah* were compared with the literature and audit trail conducted during the interview for differences and similarities.

Results

The seven participants (age 23-25 years) were female, Malay, single, final year undergraduate pharmacy students. The total duration of the interviews was 204 minutes and ranged from 21 minutes to 37 minutes and average length of 29 minutes. The variation in length was due to different student responses to questions and the requirement to ask for clarification regarding some responses. Six themes are identified and are described in Table 1 using italicised quotes from the transcripts. The participants were identified using the participant number (e.g. P6).

Individuals are to blame

Stigma was manifested by blaming individuals for 1) becoming drug addicts, 2) relapsing after treatment, 3) succumbing to peer pressure, 4) unwillingness to quit drugs or to be treated, 5) the large amount of resources required to treat addiction and its co-morbidities including human immunodeficiency virus (HIV) infections and 6) their backgrounds - they described PHSUDs as individuals with low education and coming from a dysfunctional family in rural areas. They implied that PHSUDs were unfriendly.

Moral versus biological views of addiction

Students expressed both moral and biological views and could link both components. The moral views were described in terms of addiction habit, reluctance to seek professional help, negative attitudes and desire to have short-term pleasure. The biological view was implied by knowledge regarding SUDs in terms of biological changes in the body and viewing drug addiction as a chronic disease associated with withdrawal syndromes and relapse. The biological view of addiction was linked with mental health, quality of life and attitude.

Stereotypes of unpredictability and dangerousness

Students reported that they had negative thoughts on the behaviour and personality of PHSUDs. They described them as aggressive, cheating, uncooperative, anxious and rough as well as the possibility that they might exhibit withdrawal symptoms. They were concerned as to the best way to communicate with PHSUDs given the sensitivity of some issues.

Lack of education/training among students

The students reported that their education and training in SUDs was lacking and that theory and experiential education needs to be balanced. They reported on the need to focus on the best ways to gain the trust of PHSUDs such that they are willing to engage with healthcare practitioners. They expressed concern that their peers who did not participate in the course and programme will not have the knowledge and skills to care for PHSUDs.

Lack of contact with PHSUDs

Students reported that the programme was their first encounter PHSUD patients. They described it as a positive experience where interaction with PHSUDs improved their understanding of SUDs. They perceived that PHSUDs possessed unique behaviours compared with the general public and were concerned that some of their peers refused to participate in the programme because the patients were PHSUDs. The face-to-face interactions enabled the students to gain patients' trust and to discuss and address each patient's individual needs.

Reduced stigma and increased empathy following direct contact

The students reported that their experience improved their empathy towards PHSUDs and, with a changed perception, now saw PHSUD patients in the same way they saw other patients. The exchange of information and stories during the programme provided the students with insights into the reality of their patients' struggles to quit drugs and the circumstances surrounding the development of their addiction. They expressed empathy in terms of the challenges to accessing healthcare due to stigma. They were motivated to help this population with their overall health care and to prevent relapse. Islamic values were reflected regarding smoking tobacco in that smoking may lead to other forms of addiction.

Discussion

The study provides evidence that pharmacy students hold stigmatised views of PHSUDs likely driven by pre-conceived ideas regarding SUDs common in the general population. In addition, the results provide evidence that experiential education involving face-to-face contact with PHSUDs can improve pharmacy students' views of PHSUDs and promote their interest in experiential education and didactic education regarding SUDs. The finding that pharmacy students hold stigmatised views of PHSUDs is important because stigma limits PHSUDs access to health care (Merril & Monti, 2015). Avoidance of negative labels has been reported as an important factor in encouraging individuals with SUDs to seek help from healthcare providers (Ciftci, Jones & Corrigan, 2012) and, accordingly, it is important to identify and address negative views held by healthcare professionals regarding SUDs. It has been reported that lack of education, training and support for health professionals working with stigmatised patients creates barriers, reduces engagement and

diminishes empathy (Van Boekel et al., 2013).

The finding that face-to-face interactions reduced stigma and generated empathy towards PHSUDS is important because it provides evidence that experiential education can change Malaysian students' pre-conceived stigmatised views of PHSUDs. The finding that students felt their experience should become a core component of the curriculum (e.g. involve all pharmacy students) and that more didactic education regarding SUDs should be included in the curriculum is important in demonstrating that experiential education can motivate and improve student learning. Early and continuous experiential education has been identified in an evidence-based literature review as an important component of the pharmacy curriculum (Speedie et al., 2012). Relevant to this study, though not reported directly in the published manuscript, even first-year pharmacy students have had interactions with PHSUDs in community pharmacy-based methadone clinics (Winn & Turner, 2016).

Malaysia is a Muslim country and healthcare practitioners need to be culturally competent with respect to the *Maqasid Al-Shariah* principles of basic human rights including life, health and well-being (Attum et al., 2020). Islamic teaching encourages understanding and empathy and discourages assumption and speculation. In that light, holding stigmatised views can be considered as sinful as demonstrated in the following Quranic verses:

"O you who have believed, let not a people ridicule [another] people; perhaps they may be better than them; nor let women ridicule [other] women; perhaps they may be better than them. And do not insult one another and do not call each other by [offensive] nicknames. Wretched is the name of disobedience after [one's] faith. And whoever does not repent - then it is those who are the wrongdoers" (49:11).

"O you who have believed, avoid much [negative] assumption. Indeed, some assumption is sin…." (49:12).

These verses establish one important rule which is to avoid negative suspicion as this act is regarded as sinful. This would leave the heart to be clean together with more positive thoughts towards others (Qutb, 2002). The verses also indicate that it is forbidden for one group to deride other groups as we do not know for certain which groups enjoy a better status in the eyes of God. PHSUDs may possess values known only to God despite being socially disadvantaged. With proper care and treatment, those values could be identified and nurtured as part of the recovery process. The verses also imply the right of a person not to be treated disrespectfully which occurs commonly in stigmatised populations. Islam teaches that despite their history or previous sins, people should not call others by labels that inflict pain. Islamic teaching goes even further in establishing a noble society with high standard of justice and fairness as reflected in the following Quranic verse: "And do not let your dislike of a people lead you to be unjust (5:8). In our context, this verse teaches us as healthcare professionals to maintain our standard of care to all patients regardless of their appearance or background.

Islamic teachings are against harmful practices such as substance abuse that intoxicates the mind leading to loss of self-control and prevents remembrance of Allah. However, those teachings acknowledge human limitations and require that individuals should be given opportunities to repent and correct their mistakes. People should be helped to return to normal life instead of being stigmatised and discriminated against in ways that can limit access to health care and lead to relapse. Healthcare professionals including pharmacists are responsible for delivering fair and equal services to all patients. They should care for PHSUDs with compassion and empathy to develop mutual trust and promote compliance as required by *Maqasid Al-Shariah*.

Our study suggests that higher education models should explore the potential strategies of using experiential educational intervention to eliminate stigma among current and future health professionals. We propose the Islamic guideline of good manners e.g. avoid negative assumptions, being judgmental and degrading others, not to allow hatred to cause unequal services and injustice. These components need to be incorporated into the pharmacy curriculum. Failure to overcome stigma will eventually compromise the accessibility to basic healthcare services in marginalised groups of patients resulting in poor health outcomes.

The limitation of this study included a lack of variability in the student participants due to the small sample size (other themes or sub-themes may have emerged with a larger sample size). The findings of this study cannot be generalised to other population with different characteristics. Future research should include studies with larger sample sizes and with study subjects more characteristics of the general population.

Conclusion

Pharmacy students had pre-conceived stigmatic views toward patients with a history of substance use disorders. Experiential educational was a potential strategy in promoting empathy for stigmatised patients. Experiential education upholds the principle of *Maqasid Al-Shariah* that achievement of optimal health promotes the preservation of life.

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ORIGINAL ARTICLE Open Access

Human immunodeficiency virus (HIV)/ acquired immunodeficiency syndrome (AIDS): knowledge, perception, and attitude among pharmacists in Kelantan, Malaysia.

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ABSTRACT

Introduction: Although pharmacists' role in the care of human immunodeficiency virus (HIV)-infected and acquired immunodeficiency syndrome (AIDS) patients is well established, studies had reported pharmacists' negative attitudes towards people living with HIV/AIDS (PLWHA), with negative impact on HIV management. This study aimed to explore pharmacists' knowledge of HIV/AIDS, perception towards the changing of treatment regimens of HIV/AIDS, attitudes towards PLWHA, and to identify factors affecting the pharmacists' attitudes towards PLWHA in the state of Kelantan, which reportedly have the third highest number of HIV/AIDS patients in Malaysia.

Materials and method: A validated online 43-item questionnaire was distributed to 400 pharmacists in Kelantan. Multivariate logistic regression was performed to identify factors associated with pharmacists' negative attitude towards PLWHA.

Results: A total of 170 respondents (response rate 42%) completed the questionnaire. Respondents had knowledge on the potential causes of HIV infection [median (IQR) score=13.00(1); maximum score=14.00], and preventive measures of HIV/AIDS transmission [median (IQR) score=12.00(1); maximum score=12.00]. On decision in changing regimens, the respondents agreed on the need to change treatment regimen for HIV/AIDS when required [median (IQR) score=7.00(2); maximum score=8.00]. Up to 40.6% of respondents thought that the treatment regimen should not be switched based on cost. Two-thirds of the respondents had negative attitudes towards PLWHA (67.1%). Respondents who worked in community pharmacy reported positive attitudes towards PLWHA (Adj OR=0.125; 95%CI=0.025-0.623; *p*=0.011).

Conclusion: Pharmacists in Kelantan had several misconceptions towards the causes of HIV/AIDS, preventive measures of HIV/AIDS transmission, and necessity in changing treatment regimen when required. Despite good disease related knowledge, most pharmacists had negative attitudes towards PLWHA, while pharmacists working in community settings presented more positive attitudes towards PLWHA.

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Introduction

Human immunodeficiency virus (HIV) remains a major global public health issue. Approximately 36.9 million people globally (World Health Organisation, 2019), and 87,122 people in Malaysia was living with HIV (Ministry of Health Malaysia, 2018) in 2017. Almost 33 million lives were claimed by HIV infection by 2019. However, with the increasing efforts by the healthcare sector in HIV prevention and treatment, the infection has become a manageable chronic health condition (World Health Organisation, 2019). Since 2015, Malaysia has adopted the vision of World Health Organisation (WHO) in the new National Strategic Plan in Ending AIDS 2016-2030 to end the acquired immune deficiency syndrome (AIDS) epidemic as a public health threat by year 2030, with the goal of "Three Zeros: Zero new HIV infections, zero HIV-related deaths and zero HIV-related discrimination" (Ministry of Health Malaysia, 2015).

Antiretroviral therapy (ART) is the main treatment in management of HIV infection. High levels of adherence towards ARTs of greater than 95% is needed to adequately suppress HIV-viral replication, produce long-lasting response, and halt disease progression (Paterson et al., 2000). Poor patient and health care providers relationship was found to be among one of the significant causes of poor adherence to ART (Schaecher, 2013). Involvement of pharmacists in the care of people living with HIV/AIDS (PLWHA) has been proven to be associated with better treatment outcomes through ART adherence enhancement and pill burden reduction, resulting in improved CD4 cell counts, rates of viral suppression, and reduced medication errors (Tseng et al., 2012).

HIV-related discrimination is defined as the act of treating PLWHA differently than those without HIV; while HIV stigma is defined as the negative beliefs, feelings, and attitudes towards PLWHA (Centers for Disease Control and Prevention, 2019). HIV-related stigma and discrimination is a recognised barrier in combating HIV burden. In Thailand, it was found that PLWHA were being denied health care services, provided with poor quality and delayed care, and were treated discriminatorily with violated rights due to the HIV-related stigmatization and discrimination (Pudpong et al., 2014). These negative experiences may hinder PLWHA to seek or to access healthcare services.

In Malaysia, pharmacists play significant roles in providing education and counselling to PLWHA. In clinical settings, specialised medication therapy adherence clinics run by pharmacists were set up to monitor therapy adherence and outcomes, assess medication-related issues, and provide quality services for PLWHA (Pharmaceutical Services Division Ministry of Health Malaysia, 2014). Although pharmacists' involvement in HIV-positive patients care is well-established and recognised, one study conducted among hospital pharmacists in Kedah, Malaysia reported that hospital pharmacists had negative attitudes towards PLWHA (Khan & Baig, 2013). This may indicate hindrance towards health-care accessibility of PLWHA which can further impair the Ministry of Health's efforts to curb the disease. However, these findings may not be generalised to Kelantan, a different state in Malaysia with the third most HIV case prevalence (10.3%). Distribution of HIV-positive cases were lower in Kedah (6.1%) (Ministry of Health Malaysia, 2018), and the pharmacists' exposure and awareness towards HIV may be lesser.

In addition, the population and cultural distribution of Kedah and Kelantan is very different. In terms of ethnicity groups, Kedah consists of 78% Malays, 13.6% Chinese, and 7.2% Indians; while Kelantan consists 95.6% Malays, 3.4% Chinese, and 0.3% Indians (City Population, 2017). These significant differences between the states may lead to differences in cultural beliefs which may further affect attitude towards PLWHA. Furthermore, since the initiation of WHO's National Strategic Plan in Ending AIDS in 2015, there has been increased educational activities to promote awareness and reduce HIV-stigma in the recent years (Ministry of Health Malaysia, 2015). Therefore the findings by Khan and Baig (2013) may not reflect the current scenario among pharmacists in Malaysia. Hence, this study was conducted to explore pharmacists' knowledge towards HIV/AIDS, perceptions towards changing of treatment regimen in HIV/AIDS, attitudes towards PLWHA, and investigate the factors affecting pharmacists' attitudes towards PLWHA in the state of Kelantan, Malaysia.

Methodology

Study design and study process

This cross-sectional self-administered online questionnaire-based study was conducted among pharmacists who worked in Kelantan, Malaysia. Ethical approval was obtained from the Ministry of Health Medical Research Ethics Committee (NMRR-19-26-45683). All fully registered pharmacists and provisionally registered pharmacists who practiced in Kelantan were recruited. Pharmacists who were non-Malaysian, retired, and on study leaves were excluded. The samples were included by using convenience sampling.

Emails of pharmacists who worked in Kelantan were obtained through Pharmaceutical Services Division of Kelantan with permission. Softcopies of formal invitations were distributed through valid emails to 400 pharmacists. Emails were not sent to pharmacists who had no record of emails or with invalid emails, and pharmacists who met the exclusion criteria. Two reminder emails were sent on two weeks intervals to increase the number of participants. Participation by pharmacists into this study was voluntary.

After informed consent form was signed, study participants were required to fill in respective pharmacist's registration number as a measure to ensure that only

pharmacists were included into this study, and to avoid duplication of responses. Instructions on filling the questionnaire were provided at the start of the questionnaire. All questions were displayed in the online questionnaire. For open-ended questions, participants were required to type in their answers. For closed-ended questions, participants were required to choose only one answer from the options displayed. The participants were required to answer all the questions before submitting the questionnaire. All the data were synchronised into a password-protected online subject data sheet automatically.

Study sample

Traced data from Pharmaceutical Services Division of Kelantan had shown that there were 334 pharmacists working in government facilities, 109 pharmacists working in private sectors, and 40 pharmacists working in academic institution. By using Raosoft® Sample Size Calculator, assuming the margin of error = 5%; confidence level = 95%; population size = 483; and response distribution = 50%, the minimum sample size required was 215.

Study instrument

Permission for the adoption of the validated questionnaire was obtained from Khan and Baig (2013). The questionnaire was developed into an online questionnaire. The questionnaire consisted of six sections. Compared to the questionnaire by Khan and Baig (2013), collection of demographic data were modified to include academic qualifications and place of practice, and to exclude marital status. There were no modifications done on the questionnaire from section two to section six, which consisted of 43-items. A pilot test was carried out among 20 pharmacists working in other states of Malaysia. Respondents who were involved in the pilot study were not included in this study sample.

Section one was designed to obtain the respondents' demographic information including age, gender, race, academic qualification, job experience, number of HIV encountered in the past twelve months, and place of practice. Section two was designed to assess respondents' general knowledge regarding HIV/AIDS. Three open-ended questions were included in this section, including "What does the abbreviation of AIDS stands for?", "Is HIV a transmittable disease?", and "Can HIV/AIDS be cured at this moment?".

Nominal scale "Yes/No" was applied for the respondents to answer the statements from sections three and four. Section three was designed to assess respondents' knowledge towards the causes of HIV/AIDS, which consisted of 14 items. "Yes" were the correct answers for statements one to eight. "No" were the correct answers for statements 9 to 14. One point was awarded to the respondent's score for every correct answer. Section four was designed to assess respondents' knowledge towards the preventive measures of HIV transmission, which consisted of twelve items. "Yes" were the correct answers for statements one to six. "No" were the correct answers for statements seven to twelve. One point was added to the respondent's score for every correct answer.

Section five was designed to assess respondents' perceptions on the decision in changing therapeutic regimen of HIV-positive patients, which consisted of eight items. Nominal scale "Yes/No" was applied for the respondents to answer the statements in this section. Section six was designed to evaluate the respondents' attitudes towards PLWHA, which consisted of six questions. Nominal scale "Yes/No" was applied for the respondents to answer the statements in this section. For all the statements, every "Yes" contributed one point to the respondent's total scores; while every "No" contributed two points to the respondent's total scores (Khan & Baig, 2013). The maximum possible score on this section was 12. Respondents who scored 6 were classified as having positive attitudes towards PLWHA, who scored 7-10 were classified as having negative attitudes towards PLWHA, and who scored 11-12 were classified as having very negative attitudes towards PLWHA (Khan & Baig, 2013)

Statistical analysis

Data were cleared and analysed using IBM SPSS version 20.0 (IBM Corp, Armonk, NY). Kolmogorov-Smirnov test was used to determine the normality of analysed data, with $p>0.05$ were considered as normally distributed. As all the numerical data were not normally distributed, the numerical variables were presented as median and interquartile range (IQR). Categorical data were presented as frequency and percentage. The demographic information and response statements were analysed by using descriptive statistics. Logistic regression was used to explore the independent factors contributed to attitudes of pharmacists towards PLWHA. The variables with $p<0.200$ in simple logistic regression were included into the variable selection for multiple logistic regression model. All the tests were two sided and $p<0.05$ were considered as statistically significant.

Results

A total of 170 pharmacists (79.1% of the recommended minimum sample size) completed the questionnaire with a response rate of 42%. The baseline demographic data of respondents participated in this study are summarised in **Table 1**. More than half of the respondents were aged 24-30 years old (57.1%). Majority of the respondents were females (78.8%). Most of respondents were Malay (92.4%). Almost one-third of the respondents had job experience of 4-7 years (37.1%), and almost two-thirds of the respondents

encountered 1-20 patients in the past twelve months (62.4%). Most of the respondents worked in government hospitals (61.2%).

Table 1: Demographic data of study respondents

Demographic Data (*N* = 170)	*n* (%)
Age (years)	
21-30	97 (57.1)
31-40	60 (35.3)
41-50	11 (6.5)
51-60	2 (1.1)
Gender	
Male	36 (21.2)
Female	134 (78.8)
Race	
Malay	157 (92.4)
Chinese	11 (6.5)
Indian	0 (0.0)
Others	2 (1.1)
Academic Qualification	
Bachelor	159 (93.5)
Master	11 (6.5)
Job Experience	
< 1 year	14 (8.2)
1 – 3 years	37 (21.8)
4 – 7 years	63 (37.1)
8 – 11 years	25 (14.7)
12 – 14 years	13 (7.6)
> 14 years	18 (10.6)
Number of HIV patients encountered for the past 12 months	
None	39 (22.9)
1 – 20 patients	106 (62.4)
21 – 50 patients	14 (8.2)
> 50 patients	11 (6.5)
Place of Practice	
Government Hospital	104 (61.2)
Government Health Clinic	43 (25.3)
Pharmaceutical Services Division	10 (5.9)
Community Pharmacy	9 (5.3)
Academic Institution	2 (1.1)
Private Clinic/ Hospital	1 (0.6)
Pharmaceutical industry	1 (0.6)

Pharmacists' general knowledge about HIV/AIDS

It was revealed that 87.6% of the respondents were aware of the abbreviation of AIDS, 98.8% agreed that HIV is a contagious disease, and 95.9% of the respondents understood that HIV/AIDS is unable to be cured at this moment.

Pharmacists' knowledge towards the causes and preventive measures of HIV/AIDS

Majority of the respondents aware of the potential causes [Median (IQR) score = 13.00 (1.0); maximum score = 14.00] and preventive measures [Median (IQR) score = 12.00 (1.0); maximum score = 12.00] of HIV/AIDS transmission. The respondents showed most erroneous perceptions on the causes of HIV through kissing an HIV-infected person (31.8%), followed by tattoo or body piercing (27.6%), and breast-feeding from an HIV-infected mother (26.5%) (**Table 2)**.

One-sixth of the respondents believed that treating sexually transmitted diseases (STDs) promptly did not prevent transmission of HIV (14.1%), and 10.6% of respondents believed that avoiding mosquitoes' bites can prevent transmission of HIV (**Table 3**).

Table 2: Pharmacists' knowledge towards the possible causes of HIV infection

Statements	Correctly Answered, *n* (%)
Sexual intercourse without a condom with HIV-infected person	166 (97.7)
Sharing needle with HIV-infected person	169 (99.4)
Transfusion of HIV-infected blood or receiving HIV-infected organ	169 (99.4)
Having sex with multiple sexual partners with unknown HIV status	167 (98.2)
From an HIV positive mother to her foetus	160 (94.1)
Sharing personal items such as shaving blades	141 (82.9)
Breast-feeding from an HIV-infected mother	125 (73.5)
Having tattoo or body piercing	123 (72.4)
Kissing an HIV-infected person [mouth to mouth/ French kissing]	116 (68.2)
Mosquito bites	156 (91.8)
Sharing/ eating a meal with an HIV-infected person	158 (92.9)
Using a public swimming pool	166 (97.7)
By using a public toilet	168 (98.8)
Casual contacts (hugging or touching) with an HIV-infected person	167 (98.2)

Table 3: Pharmacists' knowledge towards the preventive measures of HIV infection

Statements	Correctly Answered, *n* (%)
Avoid taking illicit drugs/use of intravenous drug	158 (92.9)
By avoiding sharing needles and syringes	170 (100.0)
Having sex with only one faithful, uninfected partner	167 (98.2)
Using condoms during sexual intercourse	167 (98.2)
Treating STDs promptly	146 (85.9)
Screening donated blood before transfusion	169 (99.4)
Not sharing swimming pools or toilet with an infected person	157 (92.4)
Not sharing food with an infected person	158 (92.9)
Isolating people living with HIV/AIDS	164 (96.5)
Do not stay with infected person in the same house	164 (96.5)
Do not have casual contact with infected person	163 (95.9)
Avoiding mosquito bites	152 (89.4)

Pharmacists' perceptions towards the decision to change treatment regimen for HIV/AIDS patients

The median (IQR) score of respondents on decision to change regimens were 7.00(2.0) [maximum score = 8.00]. Nearly all respondents agreed that treatment regimen should be changed when patient is unable to tolerate adverse reactions of the regimen (97.1%), and when HIV-viral load increases (95.3%). Two-fifth of the respondents thought that the treatment regimen should not be switched based on the factor that the patient could not afford the treatment cost (40.6%) (**Table 4**).

Pharmacists' attitudes towards people living with HIV/AIDS

Two-thirds of the respondents had negative (61.8%) and very negative attitudes (5.3%) towards PLWHA (**Figure 1**), with median (IQR) score = 8.00(3.0) [maximum score = 12.00]. Half of the respondents revealed that they were not willing to live with HIV/AIDS-positive people in the same house (51.8%). Almost two-fifth of the respondents were not willing to take care of HIV/AIDS-positive patients (41.2%). About one-third of the respondents confessed that they did not feel comfortable working together with a colleague who is HIV-positive (31.2%) (**Table 5**).

Table 4: Pharmacists' perceptions towards the decision to change treatment regimen for HIV/AIDS patients

Statements	Yes, *n* (%)
Patient's compliance is poor	154 (90.6)
Patient cannot tolerate adverse reactions of the regimen	165 (97.1)
Viral load increases	162 (95.3)
CD4 T-cell count decreases	144 (84.7)
Patient experiences opportunistic infections	143 (84.1)
Drug interactions with other medicines	160 (94.1)
Patient cannot afford the treatment cost	101 (59.4)
Patient is found to be pregnant	146 (85.9)

Table 5: Pharmacists' attitudes towards people living with HIV/AIDS

Statements	Yes, *n* (%)
Do you feel comfortable about counselling HIV/AIDS patients?	135 (79.4)
Do you feel comfortable to work together with a colleague who is a HIV/AIDS patient?	117 (68.8)
Are you willing to live with people having HIV/AIDS in the same house?	82 (48.2)
Do you feel empathetic towards people living with HIV and AIDS?	161 (94.7)
Are you willing to take care of patients who have HIV/AIDS?	100 (58.8)
Do HIV/AIDS patients deserve free treatment?	133 (78.2)

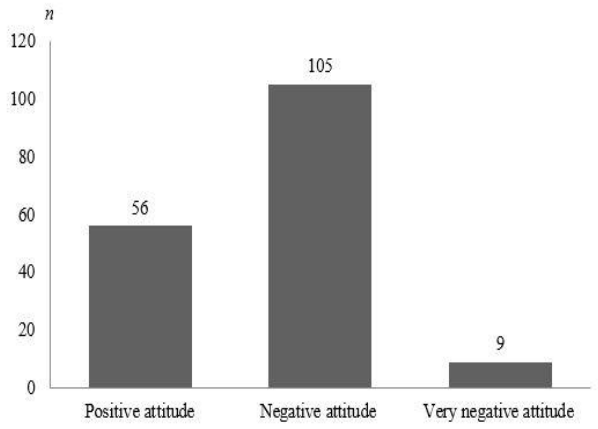

Figure 1: Pharmacists' attitudes towards PLWHA

Multivariate logistic regression was performed to identify the significant independent factors associated with respondents' negative attitudes towards PLWHA (**Table 6**). Respondents worked at community pharmacies were 0.87 times less likely to have negative attitudes towards PLWHA than respondents who practiced in other work-places (Adj OR=0.125; 95%CI=0.025-0.623; p=0.011).

Discussion

In the general knowledge assessment section, the potential of HIV transmission through kissing is the most misunderstood. This trend of misconceptions were similar to the study by Khan and Baig (2013) where up to 89.3% of respondents believe that kissing HIV-infected person contributed to HIV transmission. WHO stated that "HIV can be transmitted through the exchange of body fluids from infected people, such as blood, breast milk, semen, and vaginal secretions. Individuals cannot become infected through kissing, hugging, shaking hands, or sharing personal objects, food or water" (World Health Organisation, 2019). The hypotonicity of saliva is believed to be protective against HIV transmission by inactivating HIV-transmitting leucocytes, preventing attachment of the HIV-transmitting leucocytes to the mucosal epithelial cells, and lastly HIV production (Baron, Poast, & Cloyd, 1999).

Another highly prevalent misconception on HIV prevention among recruited respondents is that HIV cannot be prevented by treating sexually transmitted diseases (STDs) promptly. This is different from the findings of Khan and Baig (2013), where most pharmacists' misconception was avoiding taking illicit drug/ use of intravenous drug to prevent HIV transmission. Nevertheless, up to 29.3% of respondents in Kedah also believed that treating STDs promptly will not prevent HIV transmission (Khan & Baig, 2013). The risk of HIV transmission through sexual contact were reported to increase by five-to-ten folds in people with ulcerative STDs (Sahasrabuddhe & Vermund, 2007). Disruption of the integrity of epithelial mucosa by ulcerative STDs, facilitates the contact of HIV with lymphatic and circulatory systems (Cohen, 2004; Wasserheit, 1992). For inflammatory and exudative STDs, the risk of HIV transmission through sexual transmission is increased by two-to-five folds (Sahasrabuddhe & Vermund, 2007), as the infection and inflammation is associated with recruitment of urethral or cervical discharge filled with HIV-susceptible leucocytes in large volume (Cohen, 2004; Wasserheit, 1992). Management of STDs was reported to achieve reduction of 38% in incidence of HIV-infection in Tanzania over two years period (Grosskurth et al., 1995). Another study in Malawi had also shown that treatment of STDs reduced the genital tract HIV-viral loads, thereby leading to infectiousness index reduction and lowering of transmission probability (Cohen et al., 1997).

Most respondents agreed that the treatment regimen of HIV/AIDS patients should be modified only in the event of intolerable adverse reactions. Again, our finding is consistent with the earlier study conducted by Khan and Baig (2013). In instances of moderate to severe adverse events, the Malaysian Consensus Guidelines on Antiretroviral Therapy 2017 (Malaysian Society for HIV Medicine, 2017) recommend substituting the causative drug with another of the same ART class, but of different toxicity profile. In our study, we also found that the statement that most respondents disagreed on was changing of treatment regimen is necessary if the patients were unable to afford the treatment cost. In the study by (Khan & Baig, 2013), it was found that up to three-quarter of respondents did not agree to change treatment regimen on the grounds of treatment cost. However, cost of medication is a true obstacle in HIV treatment and medication adherence as reported in a qualitative study conducted in South Africa (Hardon et al., 2006). In a meta-analysis, Ivers, Kendrick, & Doucette (2005) found cost of treatment to be an important determinant of adherence towards ARTs in resource-poor settings. These studies provide insights that cost of treatment should be considered when deciding on treatment regimen. In Malaysia, HIV-positive patients who seek treatment in government healthcare facilities have free access to first-line ARTs. In the state of Kelantan, the free-of-charge treatments are extended to alternative choices or second-line treatments. Therefore, the affordable healthcare system in Malaysia may have led to the local practicing pharmacists to have such perceptions on treatment costs.

We found that two-thirds of the respondents in Kelantan had negative attitudes towards PLWHA, similar to the findings in the study by Khan and Baig (2013), which reported that a majority of the hospital pharmacists in Kedah also had negative attitudes towards PLWHA. Discriminatory attitudes of pharmacist against PLWHA were also being reported in Nigeria (Ubaka, Adibe, & Ukwe, 2014). Nevertheless, pharmacists in Iraq showed positive attitude towards PLWHA in providing care and treatment (Allela, Shareef, & Ismael, 2017). We observed that our respondents were reluctant to have social and casual contact with PLWHA, but generally had empathy towards them. Despite having high scores of knowledge in the causes and preventive measures of HIV/AIDS, and awareness that casual and social contact with PLWHA will not cause HIV transmission, the respondents were unable to apply their theoretical knowledge in daily practices towards PLWHA with negative attitudes. In fact, we found that there was no significant association between knowledge scores and attitudes towards PLWHA in this study. This finding is in contrast to another study by Balfour et al. (2010) which reported that pharmacists and health science students with better HIV knowledge were associated with lower HIV stigma in South America.

Table 6: Independent factors affecting pharmacists' attitudes towards people living with HIV/AIDS

Variables	Positive (*n*=56) *n* (%)	Negative (*n*=114) *n* (%)	Univariate Logistic Regression		Multivariate Logistic Regression	
			OR (95%CI)	*p* value	Adj OR (95%CI)	*p* value
Age (years)						
21 - 30	28 (50.0)	69 (60.5)	1.533 (0.805-2.921)	0.194	-	-
31 - 40	21 (37.5)	39 (34.2)	0.936 (0.479-1.829)	0.847	-	-
41 - 50	6 (10.7)	5 (4.4)	0.495 (0.137-1.792)	0.284	-	-
50 - 60	1 (1.8)	1 (0.9)	0.515 (0.032-8.398)	0.641	-	-
Gender						
Male	11 (19.7)	25 (21.9)	1.149 (0.519-2.544)	0.732	-	-
Female	45 (80.3)	89 (78.1)	0.870 (0.393-1.926)	0.732	-	-
Race						
Malay	50 (89.3)	107 (93.8)	1.834 (0.586-5.741)	0.297	-	-
Chinese	4 (7.1)	7 (6.2)	0.545 (0.174-1.706)	0.297	-	-
Academic Qualification						
Degree	53 (94.6)	106 (92.9)	1.333 (0.340-5.233)	0.680	-	-
Master	3 (5.4)	8 (7.1)	0.750 (0.191-2.943)	0.680	-	-
Job Experience (years)						
< 1	2 (3.6)	12 (10.5)	3.176 (0.686-14.71)	0.139	-	-
1 - 3	10 (17.8)	27 (23.7)	1.359 (0.604-3.061)	0.459	-	-
4 - 7	24 (42.9)	39 (34.2)	0.693 (0.360-1.336)	0.274	-	-
8 - 11	7 (12.5)	18 (15.8)	1.312 (0.514-3.355)	0.574	-	-
11 - 13	3 (5.4)	10 (8.8)	1.679 (0.448-6.425)	0.436	-	-
> 14	10 (17.8)	8 (7.0)	0.347 (0.129-0.936)	**0.037**	-	-

Variables	Positive (*n*=56) *n* (%)	Negative (*n*=114) *n* (%)	Univariate Logistic Regression		Multivariate Logistic Regression	
			OR (95%CI)	*p* value	Adj OR (95%CI)	*p* value
Place of Practice						
Government Hospital	28 (50.0)	76 (66.7)	2.000 (1.041-3.841)	**0.037**	-	-
Government Health Clinic	16 (28.6)	27 (23.7)	0.848 (0.408-1.764)	0.660	-	-
Pharm. Service Division	3 (5.4)	7 (6.1)	0.981 (0.236-4.078)	0.979	-	-
Community Pharmacy	7 (12.5)	2 (1.7)	0.125 (0.025-0.623)	**0.011**	0.125 (0.025-0.623)	**0.011**
Academic Institution	2 (3.5)	0 (0.0)	-	>0.999	-	-
Private Hospital/ Clinic	0 (0.0)	1 (0.9)	-	>0.999	-	-
Pharm. Industrial	0 (0.0)	1 (0.9)	-	>0.999	-	-
No of HIV patients encountered past 1 year						
None	12 (21.4)	27 (23.7)	1.138 (0.527-2.459)	0.742	-	-
1 – 20	35 (62.5)	71 (62.3)	0.991 (0.512-1.918)	0.978	-	-
21 – 50	5 (9.0)	9 (7.9)	0.874 (0.279-2.743)	0.818	-	-
> 50	4 (7.1)	7 (6.1)	0.850 (0.238-3.036)	0.803	-	-
Knowledge of HIV/AIDS [median (IQR)]	24.00 (2)	24.00 (2)	0.862 (0.703-1.056)	0.152	-	-
Knowledge on causes of HIV/AIDS [median (IQR)]	12.50 (1)	13.00 (2)	0.861 (0.641-1.156)	0.319	-	-
Knowledge on preventive measures of HIV/AIDS [median (IQR)]	12.00 (1)	12.00 (1)	0.788 (0.559-1.111)	0.788	-	-

Here, it seems that education on scientific matters may not be sufficient to achieve practices changes, and that attitudes and cultural beliefs should also be addressed in future educational programs (Reis et al.,2005).

HIV-related discriminations and its related problems is prevalent throughout the world, especially in developing countries with rich cultural, moral, and religious values - such as Malaysia. HIV/AIDS cases were often thought to be linked to moral improprieties, such as drug use, prostitution, heterosexual promiscuity, and homosexual behaviour. HIV is also believed to be highly contagious and may pose a threat to the community (Wong & Nur Syuhada, 2011). The life-threatening nature of HIV/AIDS may also lead to the negative attitudes among pharmacists towards PLWHA (Allela et al., 2017). In addition, as majority of our respondents were Malays, and up to 68.2% (n=107) of the Malays had negative attitude towards PLWHA, these negative attitudes may be associated with cultural-related conception. The burial rite of Muslim is done with bleach for known HIV-infected people, which may reinforce the societal stigma and individual anxiety towards PLWHA (Fadzil, Othman, & Mustafa, 2016). With these concerns, our respondents may refuse to take any unnecessary risks that may expose oneself in HIV transmission risk through accidental injuries or accidental transmission of infected body fluids through daily contact.

In this study, respondents working in community pharmacies had significant positive attitudes towards PLWHA. In the study that only focused in hospital pharmacists, Khan and Baig (2013) reported that 86.6% of the hospital pharmacists in Kedah had negative attitudes towards PLWHA, but the reasons for such findings were not further investigated. A study conducted among community pharmacists in India by Gupta et al. (2010) found that majority of the pharmacists had senses of professional obligation towards PLWHA and were not worried about potential HIV exposure during medications dispensing to HIV-positive patients. However, the surveyed community pharmacists were not actively involved in HIV services as about two-thirds of pharmacists revealed that they did not frequently encountered HIV-positive patients (Gupta et al., 2010). This similar situation may occur in our community pharmacy settings, where there is minimal involvement of the community pharmacists in providing care towards PLWHA, as most of the HIV-positive patients were managed in hospitals. As most of the HIV/AIDS patients with presence of opportunistic infections were treated in hospitals, this may have led to the fear of hospital pharmacists in contacting with HIV/AIDS patients compared to the community pharmacists, for example concerns for risk of exposure to contagious opportunistic infections such as occupationally-acquired tuberculosis (Engelbrecht et al., 2019).

There are some limitations and bias in this study. Some degree of social desirability bias, which may affect participants' response according to ethics of pharmacy practices, may be present in our study. The environment to complete the questionnaires were beyond our control. We were also unable to ensure that the respondents did not search for references to answer the questionnaires, or whether their responses were influenced by peers when answered in groups. A positive response bias is also likely to be present, as pharmacists with more knowledge on HIV/AIDS were more likely to fill in the questionnaire, which may yield non-representative high scores (Domnich et al., 2015). As the number of respondents included in this study were unable to achieve the recommended sample size, our results may not statistically represent the pharmacist population in Kelantan. Lastly, this study was conducted in Kelantan, which made up 10.3% of the national HIV population (Ministry of Health Malaysia, 2018); free ARTs were provided to all HIV-positive patients; lesser distribution of community pharmacies compared to other states (Pharmaceutical Services Division Ministry of Health Malaysia, 2011); and due to inherent geographical distribution of the Malay race, there were also more Malay pharmacists in the state, as reflected in the high proportion in the study respondents. Hence, the findings on this study may not necessarily be generalizable to other regions and settings in Malaysia. Further studies may also be expanded to the whole Malaysia to identify the pharmacists' knowledge, perception and attitudes towards PLWHA in Malaysia.

In the future, education and sharing sessions may be carried out to enhance the pharmacists' knowledge on HIV/AIDS, focusing on the common misconceptions. As pharmacists' high knowledge scores on causes and prevention of HIV/AIDS were not associated with the high rates of negative attitudes, it is important to investigate further the concerns of the pharmacists towards PLWHA. As stigma and discrimination reduces the quality of treatment (Yang, Zhang, Chan, & Reidpath, 2005) and affect the self-esteem of HIV-positive patients (Surlis & Hyde, 2001), efforts to assist pharmacists in overcoming their uneasiness when dealing with PLWHA will contribute positively towards future HIV disease control strategies.

Conclusion

In conclusion, several key misconceptions towards the causes of HIV/AIDS, preventive measures of HIV/AIDS transmission, and the necessity in changing treatment regimen when needed were identified among pharmacists in Kelantan, Malaysia. Despite having good disease related knowledge, most pharmacists in Kelantan present negative attitudes towards PLWHA while working in a community setting was significantly associated with more positive attitudes. Future studies should identify the causes of such

negative attitudes; and feed educational program strategies as part of the efforts to improve existing disease management measures attitudes.

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Conflict of Interest

None.

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REVIEW ARTICLE Open Access

A review on conventional and novel topical ocular drug delivery system.

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ABSTRACT

Ocular drug delivery is a very challenging area for ophthalmologists and drug delivery scientists due to the structural and barrier complexity of the eye. Barriers such as different layers of cornea, sclera, conjunctival blood flow, and tear dilution limit the efficacy of drug delivery to the anterior part of the eye in addition to more barriers present to the posterior part. Due to these, scientists have designed and studied various delivery systems to increase drug delivery and treatment efficacy to the eye. Among conventional ocular drug delivery systems, ophthalmic solution or eye drop is widely used and preferred by consumers. Conventional dosage forms available in the market are emulsion, suspension, ointment and polymeric gels. Several ocular formulations such as nano-formulations, liposomes, ocular inserts, and ocular mini-tablets are also being widely studied as future treatments to improve ocular drug delivery and as an alternative to conventional drug delivery. This review intends to summarise several conventional and novel topical formulations for ocular drug delivery.

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Introduction

Eyes are one of the important organs in a human body. As an organ of sense, it allows humans to observe and interact with their surroundings. Generally, the eyeball is divided into two parts, namely the anterior and the posterior segments. The anterior segment of the eyeball contains the cornea, iris, lens, conjunctiva, ciliary body and aqueous humour while the posterior segment consists of sclera, choroid, retinal pigment epithelium, neural retina, optic nerves and vitreous humour (Monkhouse, 2007), as illustrated in Figure 1. Even though the eyeball has many structures, only the anterior part is exposed. The remaining structure is covered and protected by the orbit in which the eyeball is situated. The small exposed portion of the eye is prone to various infections even though it has several protective mechanisms such as the eyelashes, the eyelids and tears (Tortora & Derrickson, 2015). However, eye diseases are not only limited to infections. Other diseases such as glaucoma, cataract, and allergic conjunctivitis can also inflict the eyes. Thus, a treatment is needed whenever infections or diseases are present in the eyes.

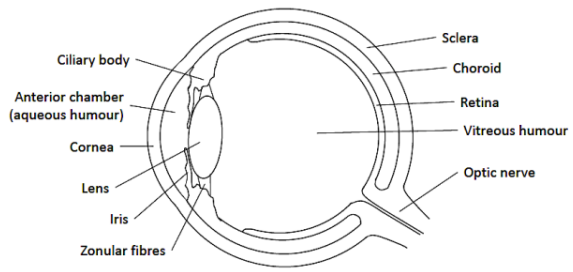

Figure 1 Human eye anatomy.

For the treatment, topical instillation of the agents is the preferable approach as it is easy, convenient and non-invasive. Eye drops are the most commonly used conventional topical ophthalmic dosage form due to the ease of administration and patient compliance (Patel, Cholkar, Agrahari, & Mitra, 2013). However, it is less effective in certain situations or treatments due to the very low ocular bioavailability and low drug permeation into the ocular tissue as a result of tear turnover, nasolacrimal drainage, and blinking. These barriers have been taken into consideration in increasing the effectiveness of the topical ophthalmic dosage form. (Souza, Dias, Pereira, Bernardi, & Lopez, 2014). As a result, various conventional and novel drug delivery systems have been developed and studied such as emulsion, suspension, ointment, and one that uses the technological development of lipid-based system and polymeric system.

Conventional topical ocular drug delivery system

Nowadays, there are several types of ocular drug delivery system in the market including ophthalmic eye drops which are highly used by patients. Others are emulsion, suspension, ointment and polymeric gel preparation.

Eye Drops

Topical eye drops are the most convenient, non-invasive and patient compliant among topical eye preparations. However, according to Pahuja, Arora, & Pawar (2012), there are a few barriers encountered by eye drops in treatments. Based on the study, a large number of patients faced difficulties in instilling the drops. Besides, the tear drainage that increases with the volume of eye drops can lead to the loss and dilution of the solution. Other than that, the amount of the drug absorbed into the ocular tissue cannot be estimated due to the limited holding capacity of the eye pocket.

Benzalkonium chloride, which is commonly used as preservative, may also cause several problems such as the peeling of the corneal epithelium cells at their borders which inhibits the growth of the cells and enlarges the intercellular spaces in the superficial cells of the cornea (Pahuja et al., 2012). Even though Ghate & Edelhauser (2006) stated that benzalkonium chloride could improve the corneal permeability of various drugs, the negative side effects caused by it should not be ignored. Due to these limitations, Patel et al. (2013) suggested the use of viscosity enhancer to increase the contact time, permeation enhancer to increase the uptake of active ingredient, and cyclodextrin as a carrier for hydrophobic molecules to increase the bioavailability of the topical eye drop.

Emulsion

The interest in using emulsion in the past has been resuscitated by submicron emulsion (ranged between 0.1 μm and 0.3 μm) with non-ionic surfactant to increase its stability (Ghate & Edelhauser, 2006). Patel et al. (2013) stated that emulsion-based formulation could enhance both the solubility and the bioavailability of ocular drugs. Generally, there are two types of emulsion that are already available in the market as a vehicle for active pharmaceutical ingredients, namely oil in water (o/w) and water in oil (w/o) emulsions (Vandamme, 2002). Among these two emulsions, (o/w) type is preferable as it is less irritating to the eye and has better ocular tolerance. According to Liang et al. (2008), emulsion-based formulation can offer several benefits in ocular formulation such that it improves the precorneal residence time, enhances the drug's corneal permeation, increases the bioavailability, and it has sustained-release properties. Precorneal residence time can also be improved by using emulsion with chitosan as its surface coating. This is based on the pharmacokinetic study done by Yamaguchi et al. (2009) using chitosan-coated emulsion in comparison to non-coated emulsion on the eyes of male albino rabbits. The results showed improvement on the emulsion mean residence time (1.5 times) and half-life (1.8 times) of the

drug in comparison to non-coated emulsion.

However, ophthalmic emulsions come with their own limitations. They have low stability and are prone to various types of instability phenomena such as flocculation, coalescence and creaming (Aldrich et al., 2013). Flocculation occurs when the dispersed phase comes out from the suspension and forms flakes. Coalescence is another instability process by which the dispersed droplets in the suspension are continuously combined to form larger droplets. Other than that, one phase in the emulsion can migrate either to the top or the bottom depending on their relative densities, forming a separated layer between the two phases known as creaming. Thus, the study suggested the use of surfactants to improve the kinetic stability of the emulsion products.

Suspension

Suspension can be defined as a dispersion of finely insoluble active pharmaceutical ingredients in a solvent (Patel et al., 2013). In other words, it is a concentrated solution of active pharmaceutical ingredients. This type of ocular drug delivery system has several benefits over ophthalmic drops. The main benefit is that it can improve the drug's contact time and duration of action due to the insoluble suspension that retains in the precorneal pocket instead of being washed away or diluted by the tear. The improvement of the duration of the drug action is also due to the different particle sizes of the suspended particles. The small particles will replenish the absorbed drug while the large particles will be retained in the precorneal pocket and undergo slow dissolution (Remington, 2011). According to Ghate & Edelhauser (2006), prednisolone acetate suspension is the most effective to go across the cornea and suppress corneal inflammation compared to prednisolone phosphate solution. There were also four weeks randomised, double-blinded, multicentre phase II clinical trial done on 1% and 2% repabimide suspension over placebo. This trial revealed that both suspensions are well tolerated and effective in treating dry eye compared to the placebo (Kinoshita et al., 2012). In addition, higher concentration of suspension was found to be more effective than one with a lower concentration.

Despite all the benefits, suspension also has several drawbacks. For example, due to the high viscosity of TobraDex®, Scoper et al. (2008) experimented by reducing the viscosity and improving its pharmacokinetics along with bactericidal activity. This resulted in a new suspension formulation, TobraDex ST®, which showed better formulation characteristics, pharmacokinetics, bactericidal characteristic and patient compliance. Another drawback of suspension formulation is that it needs to be shaken to reach the required dosage level. This will decrease patient compliance and vary the dosage of the drug delivered to the eye. Ghate & Edelhauser (2006) stated patient compliance as a limiting factor in ocular drug efficacy as the efficacy will increase with dosing frequency. With low patient compliance, the efficacy of the suspension might also be affected.

Ointment

According to Rathore & Nema (2009), ointment is a mixture of semisolid and solid hydrocarbon, such as paraffin, which is non-irritating to the eye and melts at body's physiological temperature. Commonly, there are two types of ointment, namely simple-based ointment which is made up of one continuous phase of ointment and compound-based ointment which consists of two-phase system like emulsion. When applied to the eye, the ointment will break into small drops that will remain in the conjunctival sac for a longer period of time (Baranowski, Karolewicz, Gajda, & Pluta, 2014). This action leads to the major advantage of ointment, such that it serves as a drug depot in conjunctival sac which enhances and prolongs its absorption (Ghate & Edelhauser, 2006).

According to Ali & Lehmussaari (2006), the desirable attributes of ointment development should include several factors such that it needs to be non-irritating to the eye, uniform, easily manufactured, and it does not cause excessive blurred vision. Even though it can enhance and prolong drug absorption, ophthalmic ointment faces a major drawback that can reduce its efficacy. According to Sasaki et al. (1999), the application of ointment can lead to the blurring of vision and occasional irritation, resulting in low patient compliance. Due to this, it is usually being applied at night before sleep (Rathore & Nema, 2009).

Polymeric Gel

Ocular gel is another dosage form of delivering drugs to the eye topically. Gels are made up of various materials such as mucoadhesive polymers which are important for the localised delivery of active ingredients. Mucoadhesive polymers have been used in ophthalmic gels to increase their efficacy (Shaikh, Raj Singh, Garland, Woolfson, & Donnelly, 2011). This polymer provides an attachment for the drug carrier to a biological tissue resulting in an extended contact time and an improved ocular bioavailability (Ali & Lehmussaari, 2006). There are two types of ophthalmic gels, namely preformed gel and in-situ forming gel. According to Ranch et al. (2017), ophthalmic preformed gel is less preferable as a dosage form because it is present as a gel substance at room temperature. This property has a limited use in ophthalmic drug delivery because of low accuracy and reproducibility administration of drugs, often producing blurry vision, crusty eyelids, and lachrymation. Due to this, *in situ* gels become a focus in gelling system as it provides both advantages of solution and gel.

In situ forming gel is a viscous liquid preparation that will change to a gel phase using either one of these three mechanisms which are pH triggered, temperature triggered,

or ion activated. It is preferred over the preformed gel as it is more comfortable, easily administered as a drop, and causing less to no problem to the vision (Rathore, 2010). Kaur, Singh, & Kanwar (2000) stated that good *in situ* forming gel criteria should include low viscosity, free-flowing property to be administered as a drop, and strong gel formation to withstand the sheer force of the conjunctiva. According to Gurtler & Gurny (1995), it is difficult to administer accurate dose with preformed gel due to the variation of the amount of drug delivered during administration. However, with *in situ* gel-forming formulation, it is possible to administer accurate and reproducible quantities of dose. Moreover, relatively prolonged action duration of *in situ* forming gel reduces the administration frequency and thus increases patient compliance.

Novel topical ocular drug delivery system

Even though conventional topical ocular preparations are being widely used nowadays, some drawbacks are still present in terms of their usage, efficacy and safety. Due to these, various approaches have been made and studied. One of the approaches is by utilising nanotechnology in the ocular drug delivery system through nanoparticles and nanomicelles. There are also several other approaches to improve ocular delivery system such as liposomes and ocular inserts.

Nanoparticles

Sahoo, Dilnawaz, & Krishnakumar (2008) defined nanoparticles as any particles with a diameter not bigger than one micrometer, comprising of various natural or synthetic polymers, lipids, phospholipids, or metals. There are two types of nanoparticles: nanocapsules and nanospheres. In nanocapsules, the drug is encapsulated inside the polymeric capsule while in nanospheres, the drug is uniformly dispersed throughout the polymeric matrix (Patel et al., 2013). One of the advantages of nanoparticles is that it can extend the drugs delivery to the tissues as the uptake and the distribution of nanoparticles depend on their size (Gaudana, Jwala, Boddu, & Mitra, 2009). This is proven in the study done by Sakurai, Ozeki, Kunou, & Ogura (2001) on the significance of particle size in tissue distribution. The study concluded that the smaller particle size could be distributed further to the tissue area where no large particle is present.

Many other approaches have been developed using the nanoparticle technology. One of them is solid lipid nanoparticles. Solid lipid nanoparticles have several advantages such as they can improve corneal absorption, enhance corneal ocular bioavailability for both hydrophilic and lipophilic drug, allow autoclave sterilisation, and they do not display any biotoxicity since physiological lipids are used during the preparation process (Seyfoddin, Shaw, & Al-Kassas, 2010). Other than that, solid lipid nanoparticles also show sustained drug release properties based on an *in vivo* study done by Cavalli, Gasco, Chetoni, Burgalassi, & Saettone (2002). Solid lipid nanoparticles of tobramycin showed sustained drug release for up to six hours in comparison to short duration of tobramycin eye drops with an equal dose.

De Campos, Diebold, Carvalho, Sánchez, & Alonso (2004) performed a study on chitosan fluorescent nanoparticles and found that the nanoparticles were stable upon incubation with lysozyme and did not affect the viscosity of mucin dispersion. The study found that the amount of chitosan fluorescent in cornea and conjunctiva were higher for the nanoparticles compared to the controlled chitosan fluorescent solution, and the amount was constant up to 24 hours. After 24 hours of incubation with chitosan nanoparticles, the cell survival was remarkable and the viability of the recovered cell was nearly 100 percent. Aside from that, there is also a study done by Motwani et al. (2008) on submicroscopic reservoir using nanoparticles. In this study, mucoadhesive chitosan-sodium alginate nanoparticles were used to deliver gatifloxacin to the eye. As a result, it was found that this system underwent a fast release for the first hour and continued on slow release for the rest of 24 hours study. The outcome reduces the frequency of dosing which then increases its patient compliance.

Nanomicelles

According to Patel et al. (2013), nanomicelles are the most frequently used carrier system to deliver therapeutic agents into clear aqueous solution. Nanomicelles are made up of amphiphilic molecules which are surfactants or polymers in nature that will self-assemble into micelles. There are three different types of micelles, namely regular micelles, reverse micelles, and unimolecular micelles (Trivedi & Kompella, 2010). Regular micelles are amphiphilic copolymers which self-assemble in aqueous medium while reverse micelles are amphiphilic copolymers which self-assemble in non-aqueous medium. Unimolecular micelles on the other hand are made up of the block of copolymer which has several hydrophobic and hydrophilic regions in one molecule. This enables it to self-assemble into a micelle from one molecule. Between these three types, the reverse micelle is a good candidate to encapsulate and deliver hydrophilic drug as it forms micelles with the polar part facing towards the interior covering of the hydrophilic substances. In addition, Qiu, Zhang, Yan, Jin, & Zhu (2007) mentioned that reverse micelles could also be used to encapsulate polymeric particles.

Nanomicelles pose several advantages as a drug delivery system. Nishiyama & Kataoka (2006) pointed out that it requires a simple preparation and it has the ability to improve drug solubility, lower the toxicity, raise circulation time, and increase tissue penetration with well targeted delivery properties. *In vivo* studies on rabbits done by Civiale, Licciardi, Cavallaro, Giammona, & Mazzone (2009) suggested that nanomicellar formulations are a better option for topical delivery of small molecules compared to

suspension. In another study, Cholkar, Patel, Dutt Vadlapudi, & K. Mitra (2012) concluded that nanomicellar formulations could efficiently transverse ocular tissues and deliver drug to the back of the eye tissues. However, the conventional micelles face a little drawback. It is not stable over a long period of time, having short period of sustained release, inadequate suitability for hydrophilic drugs, and system optimisation is needed for each drug (Torchilin, 2006). Thus, these need to be considered for improvement.

Liposomes

Liposomes have an aqueous core containing drug which is enclosed by one or more phospholipid bilayers. According to Patel et al. (2013), liposomes with the ability to encapsulate both hydrophobic and hydrophilic drugs can be classified into three types, namely small unilamellar vesicles (10–100 nm), large unilamellar vesicles (100–300 nm), and multilamellar vesicles (contain more than one bilayer). These liposomes are promising means in delivering ophthalmic drug due to the presence of natural phospholipids, cell-like membranes and excellent biocompatibility (Gan et al., 2013). Other than that, liposomes can attach to the hydrophobic corneal epithelium on which they constantly release the bound drug content, enhancing pharmacokinetics, and reducing the toxic side effect (Chetoni, Burgalassi, Monti, Najarro, & Boldrini, 2007). Additionally, Budai et al. (2007) stated that sustained release of the drug could be produced by using multilamellar vesicles depending on the nature of the selected lipid composition.

A study using a rabbit model was performed by Shen & Tu (2007), where they measured the concentration-time profile of ganciclovir in aqueous humour after been instilled with liposomes containing ganciclovir and ganciclovir solution. The results showed that the area under the curve for liposomes containing ganciclovir was 1.7 times bigger than the ganciclovir solution. The drug distribution of liposomal formulation was higher in sclera, cornea and vitreous humour. Another study was performed by Habib, Fouad, & Fathalla (2008) comparing fluconazole solution and fluconazole loaded liposomes on the rabbit keratitis model. After 21 days, liposomal formulation was found successful at eliminating the infection and superior to the solution. These two studies clearly show that liposomal formulation is a better delivery system than solution. However, liposomes also suffer several drawbacks. This formulation tends to be unstable, degraded, and aggregated while its fuses cause leakage of entrapped drugs during storage and after administration (Zhang & Wang, 2009). Thus, Mehanna, Elmaradny, & Samaha (2010) suggested surface modification and polymerisation to be carried out to enhance the performance of liposomes.

Ocular Inserts

Ocular inserts are sterile preparation with a thin, multi-layered and drug-impregnated solid or semisolid devices placed into conjunctival sac whose size and shape are especially designed for ophthalmic application (Kumari, Sharma, Garg, & Garg, 2010). The main purpose of the ocular inserts is to improve the contact duration between the delivery system and the conjunctival tissue to ensure a prolonged release that suits topical or systemic treatment. According to Kumar, Bhowmik, Harish, Duraivel, & Kumar (2013), there are two types of ocular inserts, namely soluble and insoluble ocular inserts. Soluble ocular inserts are generally defined as erodible, monolithic polymer that undergoes slow dissolution while releasing the drug and does not require removal from the eye. The insoluble type of ocular inserts is made up of insoluble polymer that can deliver drug by a variety of methods and at a predetermined rate, but it needs to be removed from the eye when empty.

Sultana, Jain, Aqil, & Ali (2006) viewed the delivery of ocular inserts as more controlled, sustained and continuous. In doing so, it maintains an effective drug concentration in the target tissue and minimises the number of applications. However, based on the review, they found that the usage of this delivery system is less popular among users due to physiological factors such as patients' unwillingness to abandon the traditional liquid and semisolid medication, and occasional therapeutic failures such as unintentional expulsion from the eye and membrane rupture. Ocular inserts give several advantages such that they increase contact time, exhibit prolonged release, reduce systemic side effect, reduce dosing frequency, produce accurate dosing, increase shelf-life compared to aqueous solutions and elimination of preservative, thus leave less sensitivity reaction (Kumari et al., 2010). However, ocular inserts also come with their own disadvantages. The foreign-body sensation in the eye can lead to discomfort, causing low patient compliance, excessive lachrymation which accompanies with irritation, drug dilution, and concentration reduction (Friedrich, Saville, Cheng, & Rootman, 1996). Kumari et al. (2010) also mentioned some other disadvantages of ocular inserts such as unwanted migration in the conjunctival sac, unintended loss, and difficulties to place or remove as well as interference with the vision.

Conclusion

There are various types of ocular drug delivery systems found in the literature and the market. Despite that, drug delivery remains a conundrum and major challenge for ocular and formulation scientists due to the complexity of the eye structure. Until now, topical eye drops remain the most preferred approach for eye treatment especially for the anterior application due to the ease of administration. However, eye drops formulation faced several major drawbacks that can reduce its efficacy such as loss of active agents by tear drainage, low corneal permeability, and reduced patient compliance following frequent administration. Due to these, several conventional ocular drug delivery systems have been developed as options to improve treatment efficacy. The examples are ocular

emulsion, suspension, ointment and polymeric gels. Aside from these conventional delivery systems, scientists are developing more ocular delivery systems such as nanomicelles, nanoparticles, liposomes and ocular inserts. These novel systems are developed to further increase the efficacy and safety in the application of ocular drug delivery. Despite that, there still possessed several drawbacks. It is hoped that the future novel systems would be able to overcome all the drawbacks while retains its efficacy, safety and improve patient compliance.

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Conflict of Interest

None.

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ORIGINAL ARTICLE

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Prevalence, Factors and Cost Comparison due to Potentially Inappropriate Medications (PIMs) of Elderly Outpatients in a State Hospital in Malaysia.

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ABSTRACT

Introduction: Malaysia will be a full aging nation by 2030. The elderly (aged ≥65 years old) population often has multiple comorbidities, which increases the risk of polypharmacy and potentially inappropriate medications (PIMs). This study aims to investigate the prevalence, factors associated with PIMs among elderly outpatients, and its burden of direct pharmacotherapy cost to the Ministry of Health Malaysia.

Materials and method: A cross-sectional study involving clinic prescriptions among the elderly with more than one-month prescribing duration received from a tertiary hospital specialist clinic pharmacy from March to April 2017. Patient identifiers were screened using the Pharmacy Information System (PhIS) by including prescriptions from other clinics while excluding multiple visits and duplicate prescriptions. Patients were categorised as PIM group and non-PIM groups using Beers Criteria 2015. Logistic regression analysis was conducted to examine the factors associated with PIMs. The median monthly prescription cost was compared between PIM and non-PIM groups by Mann-Whitney test.

Results: Among 472 patients, 39.4% of patients had at least one PIM while 60.6% of patients did not receive any PIM. The number of medications prescribed was an independent risk factor contributing to PIMs (OR:2.04; 95% CI:1.40, 2.97). The median monthly prescription cost for the PIM group was MYR 29.50 (≈USD 7.53) which was not statistically significant (p=0.735) compared with the non-PIM group which was MYR 28.50 (≈USD 7.28).

Conclusion: PIM was frequently prescribed in our setting with the number of medications as the only factor. However, the prescribing of PIM did not add nor reduce the direct cost of pharmacotherapy.

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Introduction

Population ageing is a phenomenon in which people live a longer life and takes place nearly in almost all countries in the world. The reduction in mortality led to an increase in population survival rate, thus increasing the proportion of elderly. The United Nations takes 60 years old and above as age reference for geriatrics. However, in most developing countries, the age of 65 is used as a cut-off as this is the age at which citizens are eligible for elderly social security payments (Guzmán, Pawliczko, & Beales, 2012). In Malaysia, the proportion of elderly aged 65 years and over had increased to 5.1% according to the National Population and Housing Census 2010, as compared to 3.9% in 2000, with a projection of increment of 0.1% per year (Hairi, Bulgiba, Cumming, Naganathan & Mudla, 2010). According to a projection by the United Nation, Malaysia will achieve the status of a full ageing nation by 2030 when 15% of the population is classified as senior citizens (Hairi et al., 2010; Abd Mutalib, Ismail & Miskiman, 2020).

Along with ageing, the body functions deteriorate and subsequently lead to multi-morbidities. To manage the symptoms and/or to treat these multi-morbidities, there is a higher risk of the usage of many medicines known as polypharmacy (Mortazavi et al., 2016). Polypharmacy is defined as the use of a large number of medications, commonly considered as five or more (Mortazavi et al., 2016). In a study in a university hospital in Malaysia, polypharmacy was further worsened with an increment in age, morbidity, usage of over-the-counter (OTC) drugs, female and the use of cardiovascular, endocrine and musculoskeletal drugs (Senik, 2006). It has been stated that patients using two drugs experience a 13% higher risk of drug-drug interactions (DDIs) and/or adverse drug reactions (ADRs), which increase to 38% when four drugs were taken and to 82% when taking seven or more drugs at the same time (Gallagher, Barry & O'Mahony, 2007). Furthermore, DDI and/or ADR were frequently misinterpreted as the onset of another medical condition in those older patients. An example of ADR is extrapyramidal symptoms (EPS) induced by metoclopramide could be misdiagnosed as the onset of Parkinson's disease, although this misdiagnosis would be less likely in young patients as Parkinson's disease is uncommon in a younger population (Kalisch, Caughey, Roughead & Gilbert, 2011). This could lead to prescribing cascade among elderly in which additional drug(s) is being prescribed to treat the DDI and/or ADR of another drug(s) which then cause polypharmacy (Hilmer, 2008). Hence, potentially inappropriate medications (PIMs) are more likely to be prescribed in this age group. Close monitoring and rational pharmacotherapy are needed when prescribing to this vulnerable group either in terms of dose, frequency or duration.

PIMs can be defined as “medications in which the risks outweigh benefits where there is a safer or more effective alternative therapy for the same conditions” (Galli, Reis, & Andrzejevski, 2016; Hefner et al., 2015). This definition of PIMs has been applied in various settings using a list of explicit criteria, such as the screening tool of older people's prescriptions (STOPP) criteria (O'Mahony et al., 2015) and Beers criteria (Fick et al.,2019). Beers criteria were first compiled by Dr Mark H. Beers, a geriatrician based on a consensus panel of experts by Delphi method on 1991, initially focused exclusively on nursing home residents. The function of the criteria is to identify potentially high-risk medications used by older people. Due to factors such as local prescribing practises and formularies, these instruments differ in their ability to distinguish PIMs in various healthcare settings. There is a systematic review on comparing applicability and sensitivity of STOPP and 2002 version of Beers Criteria. Six studies obtained were investigated and concluded that STOPP is more sensitive than the 2002 Beers Criteria (Hill-Taylor et al., 2013). However, on a more recent study shows that the 2012 version of Beers criteria identified more PIMs compared to STOPP criteria (Oliveira et al., 2015). The updated version includes drugs that should be avoided or should have their dose adjusted based on individual kidney functions and selected drug-drug interactions. It has been used widely in geriatric clinical care, education, research and in the development of quality indicators (Radcliff et al., 2015). With a long history and its development in 1991, Beers criteria were frequently updated in 1997, 2003, 2012, 2015 and most recently in 2019 by the American Geriatrics Society (AGS) (Fick et al.,2019). In contrary, STOPP criteria are only in version 2 in 2015 after its development in 2008 (O'Mahony et al., 2015).

PIMs used in the elderly is associated with an increased risk of DDIs and/or ADRs (Galli et al., 2016; Hefner et al., 2015). A systematic review conducted by Xing et al. (2019) in investigating associations between PIMs exposure and adverse events, such as ADRs, hospitalisation, and mortality. Despite no significant association between mortality and PIMs, a statistically significant correlation between ADRs and hospitalisations with PIMs was found in the combined study. A previous study shows that PIMs such as non-steroidal anti-inflammatory drugs (NSAIDs) had been associated with adverse outcomes and increase the cost of hospitalisation (Galli et al., 2016). Thus, it is crucial to identify the PIMs to reduce DDI and/or ADR in older patients. By concept analysis, deprescribing may lead to cost reduction in terms of reduced medications, reduced hospitalisation and improved adherence as having less medication to monitor (Page, Clifford, Potter, & Etherton-Beer, 2018). In the Health Economics of Potentially Inappropriate Medication (HEPIME) study among elderly aged 65 years

old and above in Germany, by controlling for the number of prescribed medicines, the gap in overall healthcare expenses between PIM and non-PIM groups was € 401 (≈MYR 1877.25, as of 2017) in a 3-month period. There is a lack of published study in Malaysia that investigates PIMs in a Malaysian public hospital and incorporating prescription cost analysis among elderly outpatients. Hence, this study aims to determine the prevalence of PIMs by using Beers criteria 2015 and the factors associated with PIMs. This study also compares pharmacotherapy cost associated with PIMs and non-PIMs.

Methods

This study was conducted in a tertiary hospital in Malaysia, Hospital Tuanku Fauziah, the only hospital in the state of Perlis at the time of the study which serves a population of 252,000 during our period of study in 2017 (Department of Statistics Malaysia, 2017). This hospital did not have in-house nor visiting geriatrician. A retrospective study was conducted among clinic outpatients aged more than 65 years old. All clinic prescriptions (prescribed for at least 4 weeks of treatment and must be taken regularly) were collected in a specialist clinic pharmacy from 1st March 2017 to 15th April 2017. The exclusion criteria were referral repeats prescriptions for patients to get the next refills in other healthcare facilities, prescriptions for intravenous and external preparation.

The patients' identifiers were further screened using the Pharmacy Information System (PhIS, Pharmaniaga®, Shah Alam, Malaysia) to include visits to other specialist outpatient clinics. If there were duplicate prescriptions of the same patients, the latest data were considered. The number of medications is defined as the number of types of medication prescribed for the patient using the latest prescriptions and considering all current clinic visits. The Beers 2015 criteria were used to identify and assess any inappropriate prescribing. Any medication categorised under PIM was checked whether it was appropriate for that patient using the PhIS system on history of medication taking and the patient medical record kept in the respective clinic. If the PIM were appropriate, it would be removed as PIM. For example, for proton pump inhibitors, they would be screened if the patient were on prolonged corticosteroids or NSAID use, or diagnosed with erosive esophagitis, Barrett's esophagitis, pathological hypersecretory condition. Two researchers examined patients' medications for PIMs used independently and any discrepancies were resolved by consensus from all researchers. The existence of polypharmacy was analysed as one of the independent variables: patients were subjected to polypharmacy when they received more than 5 medications. As pill burden is not in the scope of our study, the number of medications was calculated as the number of active ingredients for any combination drug (multiple active ingredients in a single dosage form) prescribed. Patients were divided into two groups either PIM (prescribed with at least one PIM) or non-PIM (was not prescribed with any PIM) groups.

Prior data indicated that the proportion of outpatients prescribed with at least one PIM (PIM group) was 0.276 (Lim et al., 2016). By considering type I probability error and precision both to be valued at 0.05, we needed to study 308 samples.

The data were analysed using IBM SPSS Statistics for Windows Version 20.0 (IBM Corp, Armonk, NY). For descriptive analysis, categorical data were presented as frequencies and percentage while numerical data were presented as mean and standard deviation (SD) or median and interquartile range (IQR). For inferential analysis, binomial logistic regression was used to study the covariates on the prescribing of PIM. Prescriptions cost comparison was analysed using Mann-Whitney tests as the data were non-parametric. p-values of less than 0.05 were considered as statistically significant. This study was registered with the National Medical Research Registry (NMRR-17-2668-36550) and was approved by the Medical Research Ethics Committee (MREC) Malaysia.

Results

A total of 472 outpatients were analysed in this study. 186 (39.4%) patients were prescribed with at least one PIM while 286 (60.6%) patients were not prescribed with any PIM. The mean age of patients in the PIM group was 73.8 (6.94) while for the non-PIM group was 73.0 (6.31). **Table 1** summarises patient characteristics into PIM and non-PIM groups.

Table 1: Patient characteristics (n=472)

Characteristics	PIM (n=186) n (%)	Non-PIM (n=286) n (%)
Age (years)		
65-70	82 (44.1)	127 (44.4)
71-75	38 (20.4)	72 (25.2)
>75	66 (35.5)	87 (30.4)
Gender		
Male	95 (51.1)	162 (56.6)
Female	91 (48.9)	124 (43.4)
Race		
Malay	144 (77.4)	216 (75.5)
Non-Malay	42 (22.6)	70 (24.5)
Polypharmacy		
No	69 (37.1)	156 (54.5)
Yes	117 (62.9)	130 (45.5)
Number of clinic visits		
1	181 (97.3)	276 (96.5)
> 1	5 (2.7)	10 (3.5)

Among the 472 geriatric patients, the most prescribed PIMs were diuretic, which was prescribed to 74 (15.7 %) patients followed by short and immediate-acting benzodiazepine to 55 (11.7%) patients and proton pump inhibitors to 54 (11.4%) patients, as shown in **Table 2**. A total of 278 PIMs was prescribed to 472 geriatric patients, which turns up to be 0.6 PIMs/geriatric patient. There is an average of 1.5 PIMs prescribed to 186 PIM patients.

Table 2: Types of potentially inappropriate medication (PIM) (n=278) prescribed to sample population (n=472)

PIM prescribed	PIM frequency (%)
Diuretic	74 (15.7)
Short and immediate-acting benzodiazepine	55 (11.7)
Proton pump inhibitor (PPI)	54 (11.4)
Selective serotonin reuptake inhibitor (SSRI)	29 (6.1)
Antipsychotic	21 (4.4)
Peripheral α1 blocker	20 (4.2)
Chlorpheniramine	14 (3.0)
Digoxin	5 (1.1)
Amitriptyline	5 (1.1)
Ticlopidine	1 (0.2)

Table 3: Factors associated with prescribing of potentially inappropriate medication (PIM) by logistic regression

Factors	Odd ratio	*p*-value
Age	1.02 (0.99-1.05)	0.150
Age group		
65-70	1.00 (ref.)	
71-75	0.88(0.54-1.42)	0.591
>75	1.06 (0.69-1.61)	0.803
Gender		
Male	1.00 (ref.)	
Female	1.25 (0.86-1.81)	0.236
Race		
Malay	1.00 (ref.)	
Non-Malay	0.90 (0.58-1.39)	0.636
Polypharmacy		
No	1.00 (ref.)	
Yes	2.04 (1.40-2.97)	< 0.001
Number of clinic visits		
1	1.00 (ref.)	
> 1	1.31 (0.44-3.90)	0.626

Factors being investigated were age, gender, races, the existence of polypharmacy and numbers of visit in different clinics. The number of medications was the only significant covariate ($p<0.001$): patients subjected to polypharmacy had 2.04 higher odd of having PIMs compared to patients who were not (**Table 3**). The median pharmacotherapy cost for PIM group, MYR 29.50 (≈USD 7.53, as of 2017), was not statistically significant ($p=0.735$) compared to the non-PIM group which was MYR 28.50 (≈USD 7.28). Due to this insignificance, cost of unneeded PIM was not further calculated.

Discussions

In our study, the prevalence of being prescribed PIM is 39.4%. Based on the Malaysian Elders Longitudinal Research (MeLOR) cohort study among urban community-dwelling older adults in Malaysia, the prevalence of PIM was 31.8% (Lim et al., 2017). A study in New Zealand regarding the prevalence of PIMs among elderly showed that the rate of at least one PIM being prescribed was 42.7% (Nishtala, Bagge, Campbell, & Tordoff, 2014). In a previous study from tertiary care hospital in India, showed that 29.2% of patients did have at least one PIMs (Shah, Joshi, Christian, Patel & Malhotra, 2016). A study conducted in a university medical centre in Seoul, Korea, among the 25810 outpatients, 7132 (27.6%) did have at least one PIM (Lim et al., 2016). However, the difference in the prevalence of at least one PIM might be due to the difference in study settings, availability of medications, and prescribing pattern (Abdulah et al., 2018).

The most prescribed PIMs in our setting were diuretic (15.7 %), short and immediate-acting benzodiazepine (11.7%) and PPI (11.4%). A study in India also found that the most commonly found PIMs was spironolactone (15.7%) and benzodiazepine (6.4%), while a study in Japan found out that the most common PIMs were histamine-2 (H_2) blocker (20.5%) followed by benzodiazepines (11.4%) (Akazawa, Imai, Igarashi, & Tsutani (2010). However, a study in Korea stated that the most prescribed PIMs were benzodiazepines, specifically alprazolam (11.2%) followed by clonazepam (10.8%) (Lim et al., 2016). Based on Table 4 from Beers criteria, diuretics are classified as PIM use with caution due to worsening or cause a syndrome of inappropriate antidiuretic hormone secretion of hyponatraemia. All benzodiazepines were classified as PIMs which increases the risk of cognitive impairment, delirium, falls, fractures and motor vehicles crash in older adults while proton-pump inhibitors which are classified as PIM, which increases the risk of *Clostridium difficile* infection, bone loss and fractures.

Previous studies have reported an increased risk of PIMs with age, gender, multiple medications and number of co-morbidities. A study from New Zealand showed that older age, being female and European were associated with increased risk of PIMs. In addition, reported from a study in Brazil, increasing in age and being female contributed to increasing the risk of PIMs. In contrast, a study from India and Japan found that age and sex did not contribute to PIMs. A study from Korea found that an increasing number of medications and prescribing doctors were associated

with PIM use. A systemic meta-analysis illustrated that only polypharmacy is positively associated with PIM use among the elderly, which supports the finding of our study (Santos et al., 2015). We observed a close association between PIMs and polypharmacy. This proved that polypharmacy is a factor strongly associated with PIMs as patients received a high number of medications tend to be prescribed with PIM. Furthermore, this study also found out that older age and increased number of comorbidities were associated with increased medication use (Lim et al., 2017). Hence, the result reflects the need for extra monitoring and precautions from all healthcare professionals towards elderly patients who are on polypharmacy.

In public hospitals, Malaysian citizens pay a nominal fee of MYR 5 (≈USD 1.24) for each specialist consultation visit which the cost includes the supply and refill of medication from the pharmacy (Jaafar, 2013). In our study, we only compared the median cost of medication per month based on acquisition cost, which is the direct pharmacotherapy cost on the Ministry of Health Malaysia's budget. The median cost for the PIM group was not significantly different as to the non-PIM group. There might not be many alternatives in the formulary.

Other studies on prescription cost analysis were based on mean monthly prescription expenditure (as paid by patients). In an Indian study, they identified that cost of therapy per month in the PIM group, USD 29.40 (≈MYR 118.06) was higher ($p < 0.01$) than the non-PIM group, USD 19.80 (≈MYR 79.51) (Shah, Joshi, Christian, Patel, & Malhotra, 2016).

In the study conducted in Germany, statutory health insurance covering 1/3 German population: cost for PIM patients, € 118.37 (≈MYR 554.14) was higher ($p<0.001$) than the non-PIM group, € 91.76 (≈MYR 429.57) (Heider et al., 2017). There were several limitations to our study. We did not know the exact indication for each medication that was prescribed to the elderly population. Furthermore, drugs, herbal medicine or supplements from other facilities were not considered. We did not know the exact outcomes of patients caused by PIMs.

Conclusion

This study shows that approximately one in three patients (38.9%) received at least one PIMs, with the common PIMs prescribed were diuretics, short and immediate-acting benzodiazepines and PPIs. Our study also shows that polypharmacy was the only covariate that affects prescribing of PIM(s). The prescribing of PIM did not affect the direct cost of pharmacotherapy in our setting. Pharmacists should conduct periodic medication reviews among elderly with polypharmacy, in collaboration with prescribers.

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Conflict of Interest

The authors declare no conflict of interest nor receive any external funding.

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Journal of Pharmacy

REVIEW ARTICLE Open Access

Potential Nanospray Inhalation of Remdesivir and Hydroxychloroquine using Poly (lactic-co-glycolic) Acid as Fast Delivery for Covid-19 treatment.

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ABSTRACT

Introduction: The oral medication of remdesivir and hydroxychloroquine face several limitations in covid-19 therapy. Despite having the first-pass metabolism, it also has a limitation in the patient who has hospitalised with a severe covid-19 infection. It is especially for a drug that is targeting the angiotensin-converting enzyme II (ACE2) receptor where the receptors are found abundantly in the lung, kidney, heart, and gastrointestinal tract. Therefore, an alternative delivery such as nanospray inhalation would provide a great benefit to those patients.

Methods: Scientific sources from Scopus, PubMed, Google Scholar, EBSCO, ScienceDirect, and Elsevier were accessed for publication of this review article regarding the nanospray inhalation for Covid-19.

Results: Since the main organ infected by SARS-CoV-2 is the esophagus and lung, inhalation may be the best route to deliver the drug to the site of action. It is proposed that poly (lactic-co-glycolic) acid to be used in the formulation.

Conclusion: Poly (lactic-co-glycolic) acid (PLGA) is considered a suitable polymer since it is biocompatible and noncytotoxic, it is the most widely applied in drug delivery either as carrier or excipient for the optimal formulation and distribution of the drugs. Dry powder inhalation of remdesivir and hydroxychloroquine may be an alternative way to deliver the drug against Covid-19.

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Introduction

In December 2019, the world has been threatened with a recent coronavirus outbreak caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which is believed to be originated from a seafood market in Hubei Province, Wuhan in central China (Al-Tawfiq et al., 2020; Morse et al., 2020; She et al., 2020). However, the seafood market is not the only source of the new coronavirus as 13 out of 41 cases in China were not linked to the market (She et al., 2020). This new coronavirus has caused the pandemic of pneumonia in humans (She et al., 2020; Wang et al., 2020) and causes severe respiratory failure requiring ICU admission (Bouadma et al., 2020). The disease, known as coronavirus disease 2019 (COVID-19), had been declared by the World Health Organization (WHO) as a pandemic in just 45 days from the onset of the new coronavirus emergence, indicating significant public health issues around the world (Panati & Narala, 2020). The virus not only affected global public health but also local economies (Liu et al., 2020). On January 12, 2020, the World Health Organization (WHO) had named the new coronavirus as the 2019-novel coronavirus (2019-nCoV). Disease caused by this new coronavirus was named by the WHO as Covid-19 on 11 February 2020, the same day of the Coronavirus Study Group (CSG) of the International Committee proposed to name the new coronavirus as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) (Guo et al., 2020).

As of December 31, 2019, 104 cases were diagnosed and the Covid-19 infection was extended to all the 34 districts of China including Macau, Hong Kong, and Taiwan in just 30 days. By February, the confirmed case increased rapidly to 2,175,567 with 2239 deaths reported in China, and 1151 cases were confirmed with 8 losses reported in 26 countries (Zhou et al., 2020a). Recently, the death rate of 2019-nCoV worldwide is 6.93% with 265,084 deaths reported (Covid-19 Visualiser) compared to 9.6 % of SARS-CoV and 34.4% of Middle East Respiratory Syndrome Coronavirus (MERS) (She et al., 2020). As of May 12, 2020, the number of deaths worldwide is 303, 371 (Covidvisualizer.com). It is estimated that the basic reproductive figure for Covid-19 infection lies between 2.2 and 4.8 (Bouadma et al., 2020; G. Zhou et al., 2020). Thus it is believed that the increase in the widespread of SARS-CoV-2 infection is due to genetic variation and regular recombinant of the viral genomes as well as increased human and animal activities (She et al., 2020).

Generally, coronaviruses (members of the family *Coronaviridae*) are RNA viruses with features of enveloped, single-stranded and positive-sense, (Guo et al., 2020; She et al., 2020), which able to spread to birds and all mammals including humans, thus affecting the body systems such as respiratory, enteric, hepatic, and neurologic (She et al., 2020). It was reported that the genome sequence of SARS-CoV-2, SARS-CoV, MERS-CoV is 96.2%, 75-80%, and 40% similar to the beta-coronaviruses detected in bats respectively (Guo et al., 2020; She et al., 2020; Wang et al., 2020). The SARS-CoV and MERS-CoV are both fatal coronaviruses that occurred in different areas in 2002 and 2012 respectively (She et al., 2020). Meanwhile, SARS-CoV-2 shares 82% similarity of the sequence identity with the SARS-CoV (Morse et al., 2020; Wang et al., 2020). Because of the comparable genomic sequence, the bat was allegedly to be the natural host of the virus originated in which the virus is spread by an unknown intermediate host from bats to humans (Guo et al., 2020).

In the early of the pandemic, remdesivir and hydroxychloroquine have been suggested as possible prevention or treatment of covid-19 based on evidence of *in vitro* inhibition of SAR-Cov-2. The current available formulation of remdesivir and hydroxychloroquine are not suitable for critically infected patients, therefore, we would like to provide a brief insight regarding an alternative preparation for critically ill patients.

Methods

Information on the nanospray inhalation for Covid-19 was obtained *via* a literature search conducted for publications using various electronic databases, such as PubMed, Google Scholar, International Islamic University Malaysia IIUM EBSCO, ScienceDirect, and Elsevier. The publications selected for this review is ranging from 2000 to 2020 to make sure the information gathered is validated and updated. The keyword 'SARS-CoV-2' and 'Covid-19' were used as primary searches, while terms such as nanospray, inhalation, and antiviral were applied as secondary searches. As there was a limited source on *in-vivo* and *in-vitro* studies related to nanospray inhalation for Covid-19 at the time of writing this article, the only scientific theory regarding nanospray inhalation from the selected literature search was included to highlight the therapeutic use of nanospray inhalation for Covid-19. Literature search on potential medications to be delivered as nanospray inhalation such as remdesivir and hydroxychloroquine were also included. Therefore, this review article is not exhaustive to medications used for Covid-19 that cannot be delivered through nanospray inhalation technology.

Transmission and Clinical Presentation

As mentioned before, Covid-19 is a disease that is spread from an unidentified animal source to a human and it is transmissible from one person to another (Bouadma et al., 2020). SARS-CoV-2 is transmitted primarily through respiratory droplets generated after coughing, sneezing, and speaking (Itani et al., 2020) as well as through direct contact with an infected person for a low infective dose

(Guo et al., 2020). The symptomatic or asymptomatic infected person may transmit the SARS-CoV-2 to a healthy person who is in close contact, or when he or she touches an infected surface and subsequently touches his or her face especially at the area of eyes, nose, or mouth (Itani et al., 2020). Family members, such as relatives and friends having in direct contact with patients or incubation carriers, are at high risk of infection transmission (Guo et al., 2020). Although the SARS-CoV-2 droplets can move less than 2 meters and do not stay in the air, a study found that the SARS-CoV-2 can remain sustainable in the aerosols for up to 3 hours, and on copper, cardboard as well as on plastic or stainless steel for 4 hours, 24 hours and, 2 to 3 days respectively (Itani et al., 2020).

The incubation period is mainly between 3 to 7 days (within 14 days) (Guo et al., 2020; Itani et al., 2020; She et al., 2020) and it is contagious during the latency period (Guo et al., 2020). There are no specific symptoms but the most frequent ones are an increase in body temperature, cough, exhaustion, sputum production, breathlessness, sore throat, and headache (Guo et al., 2020; She et al., 2020). Some patients may experience gastrointestinal symptoms (diarrhea and vomiting) (Guo et al., 2020). If the disease is uncontrolled, it can develop severe complications including acute respiratory distress syndrome (ASDS), septic shock, multiple organs failure, or even death (Guo et al., 2020; Itani et al., 2020; She et al., 2020). The most susceptible people to severe diseases are older patients (>65 years old) and people with comorbidities such as hypertension, chronic obstructive pulmonary disease, diabetes, and cardiovascular disease (Guo et al., 2020; Itani et al., 2020).

Site of Action

It was found that the SARS-CoV-2 multiplies better in epithelial cells of the primary human airway than in normal tissue-culture cells as compared to the SARS-CoV and MERS-CoV (She et al., 2020) and it utilizes angiotensin-converting enzyme- 2 (ACE-2) receptor **(Figure 1)**, just like SARS-CoV, to infect human being (Guo et al., 2020; She et al., 2020; G. Zhou et al., 2020). ACE- 2 can be found in type II alveolar cells of the lung and other epithelial cells located at the heart, kidney, ileum, esophagus, and bladder (Zhou et al., 2020). The receptors are abundant in lung alveolar epithelial cells and enterocytes of small intestines (Guo et al., 2020). The coronaviruses use their spike protein to attach to a host cell receptor for entry (Morse et al., 2020). Structural analysis showed that the spike of SARS-CoV-2 glycoprotein has a higher binding affinity towards the ACE-2 receptor (Morse et al., 2020; G. Zhou et al., 2020). The S-glycoprotein of the SARS-CoV-2 has 2 subunits; S1 and S2. S1 is responsible for evaluating the number of virus-hosts and cell tropism with receptor-binding domain (RBD), while S2 is responsible for the fusion of virus into cell membrane through a pair of domains, heptad repeats 1 (HR1) and (HR2) (Guo et al., 2020). Besides that, the SARS-CoV-2 also requires two proteins which are coronavirus main proteinase (3CLpro) and the papain-like protease (PLpro) to mediate the formation of new virions through the proteolysis process and one protein which is replicate or RNA-dependent RNA polymerase (RdRp) to replicate once it enters the human epithelial cells (Morse et al., 2020).

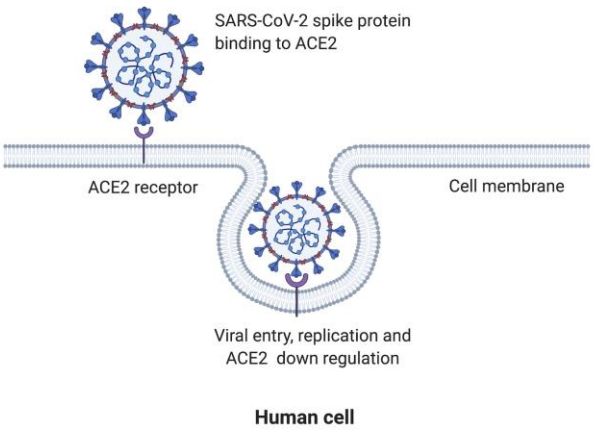

Figure 1. Typical binding of SARS-CoV-2 to ACE2 receptor. ACE2 receptor is an entry point by the virus to propogate inside the human cells.

Treatment Approaches

Currently, there is no definite treatment or vaccine available in the market to kill the SARS-CoV-2 (Bouadma et al., 2020; Panati & Narala, 2020; She et al., 2020; Wang et al., 2020). In fact, in clinical practices, supportive therapy, oxygen therapy, or mechanical ventilation are used to prevent complications or reduce symptoms of the Covid-19 disease associated with respiratory disorders (She et al., 2020). It was reported that the SARS-CoV-2 invades the human body through its entry via ACE-2 receptors which are expressed on the epithelial cells of the lung, intestines, kidney, and blood vessels (Panati & Narala, 2020). Targeting directly the ACE-2 receptors using ACE inhibitors or angiotensin-receptor antagonists had led to high morbidity in a patient with diabetes and hypertension as the drugs promote overexpression of the ACE-2 receptors, making them more favorable situation for the virus to invade the body system (Gaurav & Ramarao, 2020; Panati & Narala, 2020). Due to that, several drugs and treatment options were undergoing clinical trials and being tested for their efficacy against Covid 9 disease. Among them, remdesivir (an experimental drug) and hydroxychloroquine are the most frequent drugs that have been tested extensively (Gaurav

& Ramarao, 2020) due to their efficient action in impeding SARS-CoV-2 infection *in-vitro* (Morse et al., 2020).

Remdesivir, a novel antiviral drug, and chloroquine, an anti-malarial drug, were found to be effective in preventing the current novel coronavirus at an effective concentration (EC) of 1.1 µm (Colson et al., 2020). Remdesivir is a prodrug and an analogue of a nucleoside. It was produced by Gilead Sciences located in the United State of America (USA) (Liu et al., 2020). In the earliest case report, the first Covid-19 patient from the USA had shown an improvement in his clinical condition after being treated with intravenous remdesivir (Guo et al., 2020; Holshue et al., 2020; Liu et al., 2020; She et al., 2020). To block the Covid-19 infection, remdesivir may inhibit the viral receptor-binding domain (RBD)-ACE-2 interaction by using peptides and their cocktails derive from RBD and ACE-2 (Morse et al., 2020). Meanwhile, chloroquine was commonly used to treat malarial infection and autoimmune disease, but nowadays, it has been reported that this drug has a wide-spectrum activity against viral infection. It can prevent virus infection by raising the endosomal pH necessary for virus and cell fusion, as well as obstructing the glycosylation of cellular ACE-2 receptors by SARS-CoV (Wang et al., 2020).

Since ACE-2 receptors are abundant in lung epithelial cells and its associated with respiratory problems among infected patients with SARS-CoV-2, this article review focuses to target directly the lungs through localised therapy by using nanospray inhalation. This formulation can produce an ideal particle size of a drug (about 1-5 µm) which can efficiently reach the deep lung, allowing its penetration and deposition in the alveolar (Arpagaus et al., 2018; Karathanasis et al., 2005). This formulation is also able to avoid the first-pass metabolism and allow for more rapid onset of therapeutic action (Bartolucci, 2017). Thus, the lung which is infected by SARS-CoV-2 would be more reachable with pulmonary delivery compared to oral and intravenous injection. Besides that, poly(lactic-co-glycolic acid) (PLGA) will be used as a polymer since it is biocompatible, noncytotoxic, and widely used as carrier or excipient in drug delivery to achieve the best formulation and delivery of the drugs (Arpagaus, 2019a).

Properties and Clinical Trials of Remdesivir and Hydroxychloroquine

Remdesivir

Remdesivir (GS-5734), is an investigational nucleoside analog prodrug that has not yet approved or licensed anywhere including by the Food and Drug Administration (FDA) of the United States (US) (Al-Tawfiq et al., 2020; Guo et al., 2020; Ko et al., 2020). It is a small molecule with a molecular weight of 602.6 g/mol and its chemical formula is $C_{27}H_{35}N_6O_8P$ (Ko et al., 2020). It was manufactured by Gilead Sciences in 2017 to treat the Ebola virus that was occurred in 2016 (Al-Tawfiq et al., 2020; Eastman et al., 2020; Gordon et al., 2020). Besides, it was reported that remdesivir can treat various RNA viruses including coronaviruses like SARS-CoV and MERS-CoV which were discovered in 2017. Currently, it has been investigated as one of the promising medications for SARS-CoV-2 infection (Eastman et al., 2020). This drug demonstrated wide antiviral activities such as inhibit human and zoonotic coronavirus (including SARS-CoV-2 and Ebola virus) *in vitro* and produce preventive and therapeutic effects in animal models of MERS-CoV and SARS-CoV infections (Zhou et al., 2020).

Inside the cell, the prodrug remdesivir (GS-5734) is metabolised into adenosine nucleotide analogue (GS-441524). Since the nucleotide analogue, GS-441524 is not highly cell-permeable, it requires di- and tri-phosphorylation to generate active nucleoside triphosphate (NTP) (Eastman et al., 2020; Ko et al., 2020; Sheahan et al., 2017). This is because the initial phosphorylation of nucleotide analogue into monophosphate form, is quite polar, so it could not diffuse back through the cell membrane and being trapped in the cells (Eastman et al., 2020). The NTP then will be misintegrated into the RNA viral via the viral RNA-dependent RNA polymerases (RdRp) during genome replication thus suppressing viral RNA synthesis (Eastman et al., 2020; Gordon et al., 2020; Ko et al., 2020). The NTP resembles adenosine triphosphate (ATP) (Gordon et al., 2020). The NTP confuses the viral RdRp by working as an incorporation competitor with adenosine triphosphate (ATP) to enter into the nascent chain of viral RNA and results in immature cessation (Wang et al., 2020).

A case report associated with the earlier confirmed case of Covid-19 in the United States (US) has proven the efficacy of remdesivir to combat SARS-CoV-2. Intravenous remdesivir was given on the 7th day to an infected Covid-19 patient following reports on the development of severe pneumonia. Surprisingly, the patient's medical status showed a positive outcome on the following day with no observed adverse effects. This finding has encouraged other researchers to conduct several clinical studies for the use of remdesivir in dealing with Covid-19 disease (Holshue et al., 2020). On the other hand, three out of twelve positive Covid-19 patients had been given with remdesivir treatment for 4-10 days, in which 200 mg IV was administered on the first day, and 100 mg for each subsequent day. It was noted that all the treated patients encountered "transient gastrointestinal symptoms, such as nausea, throwing up, gastroparesis, or rectal bleeding" after the initial dose. However, the therapy proceeded until there was an amelioration in the respiratory symptoms. Nevertheless, this finding is omitted from clinical effectiveness or health review due to the limited number of sample study involvement and lack of controlled randomisation (Eastman et al., 2020).

In China, a phase 3 randomized, quadruple-blind, placebo-controlled clinical trial was enrolled at Capital Medical University, to determine the safety and efficacy of remdesivir in 308 hospitalised and infected adult patients with manifestations of mild to moderate symptoms of Covid-19 infection (NCT04252664). On the next day, another phase 3 clinical trial (NCT04257656) was enrolled at the same university to measure the efficacy and safety of remdesivir in 452 hospitalised patients with severe SARS-CoV-2 respiratory disease. All the participants involved in both trials were randomly administered with either placebo or remdesivir 200 mg loading dose on the 1st day followed by 100 mg intravenous once-daily as maintenance doses for the following 9 days. After 28 days of both treatments, the patients' conditions such as fever, oxygen saturation, and respiratory rate became normal and there was also alleviation of cough for about 72 hours (Al-Tawfiq et al., 2020; Eastman et al., 2020).

To further evaluate the safety and efficacy of remdesivir, several phases 3 clinical studies were launched on infected Covid-19 patients around the world. Among the studies are NCT04292899, NCT04292730, NCT04280705, NCT04323761, ISRCTN83971151, NCT04315948, 2020-001052-18, NCT04321616, NCT04314817, NCT04302766 (Eastman et al., 2020) and NCT04252664 (Itani et al., 2020). However, since remdesivir is an experimental drug, it would be insufficient to be used for dealing with a huge number of patients promptly. Thus, another choice of potential drugs for large-scale use is chloroquine as it is largely available with proven safety record as well as relatively low cost (Liu et al., 2020).

Chloroquine (CQ) and Hydroxychloroquine (HCQ)

Chloroquine (4-aminoquinoline) is known as a drug used to treat malarial infection and autoimmune disease, and now, has been reported as a possible antiviral drug with broad-spectrum activity (O'Neill et al., 1998; Wang et al., 2020). It was synthesised by Hans Andersag, in 1934, under German chemical and pharmaceutical company Bayer AG (Thomé et al., 2013). It is a safe and cheap drug that has been used for more than 70 years (Gao et al., 2020; Wang et al., 2020). CQ with the chemical structure $C_{18}H_{26}CIN_3$ is a small molecule and has a molecular weight of 319.872 g/mol (O'Neill et al., 1998). In 1946, the first derivate of CQ, hydroxychloroquine (HCQ) sulfate was synthesised by inserting a hydroxyl group into chloroquine and exhibited lesser (~40%) toxic than chloroquine in animals (Liu et al., 2020; O'Neill et al., 1998). Both drugs, CQ, and HCQ are widely available as anti-inflammatory agents for treating rheumatoid arthritis and lupus erythematosus (Gao et al., 2020; Liu et al., 2020; Moore, 2020). These drugs are weak base drugs with similar chemical structures (Liu et al., 2020) and mechanisms of action as antiviral, anti-inflammatory, and immunomodulator. Thus, chloroquine and hydroxychloroquine may effective in treating patients with COVID-19 pneumonia (Gao et al., 2020; Wang et al., 2020).

Both CQ and HCQ interfere with the fusion of SARS-CoV with the ACE-2 receptors on cells via de-acidification of lysosomes, thereby preventing cathepsins that require acidic conditions for optimal SARS-CoV spike protein cleavage (Gao et al., 2020; Liu et al., 2020; Singh et al., 2020). Originally, chloroquine (CQ) is lipophilic and in unprotonated form. It diffuses passively through cell membranes and into endosomes, lysosomes, and Golgi vesicles; where it becomes di-protonated (doubly positively charged), trapping the chloroquine in the organelle and elevating the surrounding pH. The resultant increased in the pH value of the endosomes prevents viral replication including fusion and uncoating (Colson et al., 2020; Thomé et al., 2013; Warhurst et al., 2003). CQ and HCQ do not affect the level of ACE-2 expression on cell surfaces, but instead, they prevent the viral entry by inhibiting terminal glycosylation of ACE-2 receptor (target receptor for SARS-CoV and SARS-CoV-2 to enter the body), making it less efficient to interact with the SARS-CoV-2 spike protein (Thomé et al., 2013).

A group of researchers from China had previously conducted an *in-vitro* study to compare the antiviral effect of HCQ and CQ on VeroE6 cells infected with SARS-CoV-2 infection. The outcome showed that both drugs can efficiently inhibit COVID-19 infection (by reducing viral replication) with conventional dosing because of its high distribution in tissues as well as in the lung. They inhibited the entry, transport, and post-entry of SARS-CoV-2 into the cells. It was also reported that HCQ is less potent and less toxic than CQ in combating SARS-CoV-2 (Liu et al., 2020; Singh et al., 2020). This is because the hydroxyl group in the HCQ that makes it less permeable to the blood-retinal barrier thus lessen the risk of retinal toxicity (Singh et al., 2020). However, HCQ has a low selectivity index (SI) as compared to CQ thus, careful designing and safe management of the SARS-CoV-2 infection is required (Liu et al., 2020).

Besides, *in vivo* evaluation using infected SARS-CoV-2 VeroE6 cells had been conducted to assess the effects of seven potential drugs including remdesivir and CQ on the cytotoxicity, virus production, and the rate of virus infection. At low-micromolar concentration, both remdesivir (EC_{50}: 0.77 μM, EC_{90}: 1.76 μM) and CQ (EC_{50}: 1.13 μM, EC_{90}: 6.9 μM) effectively hindered the SARS-CoV-2 infection. It was known that remdesivir blocked at post-stage virus entry meanwhile chloroquine blocked both entry and following-entry of the SARS-CoV-2 infection in the VeroE6 cells. The antiviral activity of CQ is also enhanced with the synergy effect of its immune-modulating activity (Wang et al., 2020). Interestingly, in

another study, it has been reported that the serum level of HCQ sulfate in humans is between 1.4-1.5 µM when given at doses of 6-6.5 mg/kg/day. This suggests that high distribution and concentration of HCQ sulfate in the liver, spleen, kidney, and lung could be achieved to inhibit SARS-CoV-2 infection (Liu et al., 2020).

To evaluate the efficacy and safety of CQ and HCQ, several ongoing clinical trials were currently conducted. The clinical trials can be tracked by the following numbers: NCT04303299, NCT04303507, NCT04304053, NCT04304053, NCT04286503, NCT04307693, NCT04261517, and NCT04308668 (Singh et al., 2020).

Nanospray Inhalation

The coronaviruses use their spike protein on its surface to recognise and bind to the angiotensin-converting enzyme (ACE)-2 receptor (abundant in the lung epithelial cells) of the host cell causing respiratory tract infection (Yang & Wang, 2020). This gives the idea that local delivery of inhaled drugs which is usually used to treat lung diseases such as asthma, chronic obstructive pulmonary disease (COPD), and cystic fibrosis (Ungaro et al., 2012) may be necessary to treat SARS-CoV-2 infection. Lungs offer a unique route for localised therapy because of the large surface area of the alveolar region, the equivalence of inhalation to arterial injection, reasonably low proteolysis activity in the alveolar space, and lack of first-pass hepatic metabolism (Karathanasis et al., 2005; Ungaro et al., 2012). Inhalation of powder particles with a size below 5 µm can be directly and efficiently delivered into the lower part of the respiratory tract (Ahmad et al., 2015; Beck-Broichsitter et al., 2012; Karathanasis et al., 2005; Ungaro et al., 2012). This local type of treatment provides fewer systemic effects than oral therapy (Ungaro et al., 2012).

Spray-dried powder is a suitable dosage form for the delivery of drugs through inhalation. It can be produced by a spray drying technique which has been an important technique for the pulmonary delivery of pharmaceutical proteins (Haggag & Faheem, 2015). Spray drying is one-step, rapid, reproducible, and scalable solvent evaporation system that is suited to convert a solution, suspension, or emulsion into controlled size and shape of dried powder particles (Arpagaus, 2012, 2018, 2019b) **(Figure 2)**. It is widely used in the pharmaceutical sectors for processing excipient, microencapsulation, or granulation (Arpagaus, 2018). There are two types of spray dryers available in the market nowadays; standard spray dryer and nanospray dryer. A standard spray dryer uses a pneumatic nozzle system while a nanospray dryer uses a piezoelectric system to produce liquid feed droplets by spraying the starting liquid material containing the drug into a hot drying medium (Patrizia et al., 2014). A standard spray-drying procedure comprises of four steps which are 1) atomisation of the liquid feed, 2) evaporating of the liquid feed through the drying gas, 3) formation of a particle, and lastly 4) separation and collection of the formed particle (Arpagaus, 2019b; Schafroth et al., 2012).

Interestingly, the innovative nano spray dryer has become a more promising technology to produce powder particles for inhalation as compared to standard spray dryers because of its ability in controlling and reducing powder particles (Patrizia et al., 2014). Nanospray dryer could produce liquid droplets using a piezoelectric system that functions at a certain ultrasonic frequency (60 kHz) and thus forming a mist of droplets that has a remarkable ultra-fine particle size. The final dried particles eventually have an electrostatic charge which enables them to be deposited on the surface of the collecting electrode (Arpagaus, 2012; Schafroth et al., 2012). The uniqueness about this new spray dryer is that it utilises a gentle laminar airflow to minimise sample loss. Besides, it has a spray head device consists of a stainless-steel membrane. This membrane has several specific micron-sized holes (spray meshes with hole sizes of 4.0, 5.5, or 7.0 µm) to generate small particles (size ranging from 3 to 15 µm) in a very narrow size distribution. Last but not least, the novel spray dryer has an electrode collector that collects particles electrostatically charged to produce high yields and thus reduce particle wastages (Arpagaus, 2012; Patrizia et al., 2014; Schafroth et al., 2012).

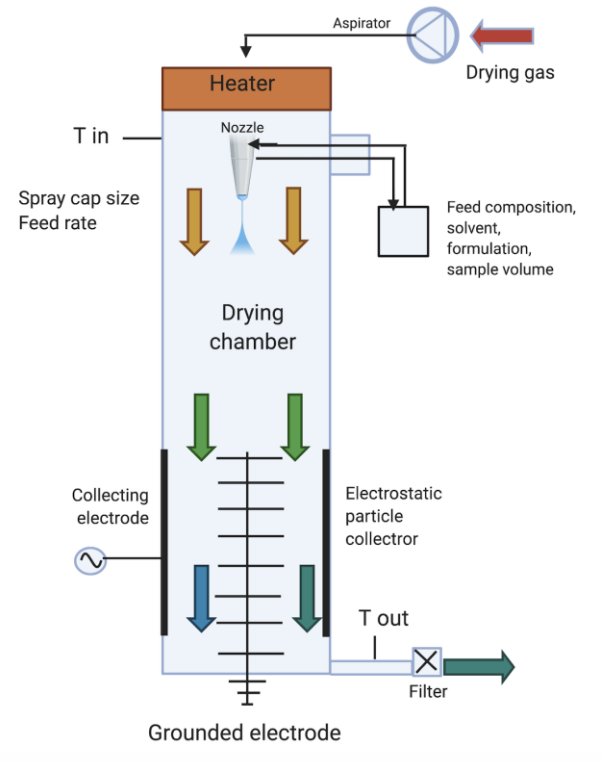

Figure 2. Principle operation of Nanospray drier (Adapted from Arpagaus, 2012)

The advantages of nanospray drying are 1) particle size and shape optimization for pulmonary drug delivery, 2) high bioavailability of the drug in the lungs as compared to oral administration, 3) reduction of drug doses, 4) minimizing systemic side effects, and 5) increases patient adherence (Arpagaus, 2012, 2019b; Arpagaus et al., 2018). Besides that, nano spray drying is suited for heat-sensitive drugs and various excipients (Haggag & Faheem, 2015). It also has been used to encapsulate both hydrophobic or hydrophilic drugs, peptides, and proteins into particles for controlled drug release (Swider et al., 2018). A reasonable size range (approximately 0.5-3.3 um) can also be achieved especially for locally acting inhaled drug particles (Arpagaus & Meuri, 2010). An amount of powder about 30 mg can be collected from the electrostatic particles collector thus allowing economical uses of expensive pharmaceuticals ingredients. Small sample quantities of about 5 to 10 mL are also possible to be spray dried with the help of a nanospray drying technique (Arpagaus, 2012, 2019b).

Proposed Strategies

There is a high expectation of using remdesivir and hydroxychloroquine as an effective treatment against SARS-CoV-2 infection due to its promising anti-SARS-CoV-2 activity; antiviral, anti-inflammatory, and immuno-modulator. A targeted administration of nasal HCQ has been reported to reduce histamine and leukotriene release in one of the animal studies with no significant acute toxicity (Barrett et al., 2008), and in cell cultures and animal model, remdesivir has shown the inhibition of replication of coronavirus. Besides, its proven safety profile and low price in the market make it a drug of choice to be produced on a large-scale amid the SARS-CoV-2 pandemic outbreak. Nanospray inhalation of remdesivir and hydroxychloroquine might be the best option to deliver the drug quickly to the lung epithelial cells particularly, the alveolar regions, where ACE-2 receptors are mostly located.

The ability of a drug to deposit and retain on the lung epithelial cells, and to overcome extracellular and cellular pulmonary barriers are essential factors to be considered in the design of inhaled powder particles (Ungaro et al., 2012). The capability of the powder particles to deposit on the lung relies on its aerodynamic diameter (D*aer*) while rapid macrophage uptake and clearance of noxious particles (including solid drug particles) depend on the D*geo*. To get to the deep lung, powder particles should have a D*aer* and D*geo* below 5 µm. However, the 5 µm of geometric diameter (D*geo*) of the particles can promote aggressive pulmonary macrophage activity, thus resulting in a brief period of delivery for an inhaled drug (Karathanasis et al., 2005). In contrast, nanoparticles with a size <1 µm will have difficulty to deposit at the pulmonary airways as they are mostly will be exhaled after inhalation (Beck-Broichsitter et al., 2012).

To overcome this disadvantage, it is highly recommended to utilise polymeric drugs that are easy to control and target their delivery to a specific region especially the lungs. A polymer such as biodegradable poly(lactic-co-glycolic-acid) (PLGA) is the most frequently used as a carrier-mediated lung targeting for the delivery of inhalation particles (Ungaro et al., 2012). PLGA co-polymer consists of polylactic acid (PLA) and polyglycolic acid (PGA). It can be easily hydrolysed by body fluids into non-toxic metabolites monomers, lactic acid, and glycolic acid causing minimal systemic toxicity (Arpagaus, 2019b; Bartolucci, 2017; Danhier et al., 2012; Essa et al., 2020; Pandey & Jain, 2015; Swider et al., 2018). It is available for commercial use and licensed by the United States Food and Drug Administration (US FDA) and the European Medicine Agency (EMA) at different molecular weights and lactide/glycolide ratios (Bartolucci, 2017; Danhier et al., 2012; Hirenkumar & Steven, 2012; Pandey & Jain, 2015). Interestingly, PLGA copolymer can be stored in powder form for a lengthy period (Swider et al., 2018). Several studies found that nanospray dried PLGA particles could produce from approximately 2 µm to below 200 nm spherical powder particles (Arpagaus, 2019a) which is within an ideal nanoparticle size for use in dry powder inhaler (DPI) (Bartolucci, 2017; Beck-Broichsitter et al., 2012).

By applying the nanospray drying technique, any hydrophobic or hydrophilic active ingredients can be encapsulated in polymeric wall materials to improve the drug formulation and to protect and deliver the drug to the correct site and time in the body (Arpagaus, 2018). Generally, encapsulated nanoparticles are described as solid particles (size less than 1 µm) suspended in a liquid medium. The smaller the size of the nanoparticles, the larger their surface area. Subsequently, the dissolution and absorption rate of the nanoparticles is improved, and thus, lead to the high bioavailability of the encapsulated drug (Arpagaus, 2019b). The common method for PLGA-based nanoparticles formulation that is suitable for hydrophobic drugs is the emulsification-solvent evaporation technique (Danhier et al., 2012; Hirenkumar & Steven, 2012). In this technique, an organic solvent like dichloromethane will be used to dissolve both polymer and the desired drug. After that, the polymer solution will be mixed with water and a surfactant such as polysorbate-80 or poloxamer-188 to form an oil in water (O/W) emulsion (Danhier et al., 2012). The mixture then is atomised into hot gas to evaporate the solvent. The resulting dried particles that contain the drug will be dispersed in an amorphous polymer matrix with a geometric mean diameter of the particles of about 1-2.7 µm and a mean mass diameter of <4 µm, (using 4 µm spray mesh size) (Arpagaus, 2018). The size range is ideal for the delivery of the drug to the lower respiratory region.

Several formulation parameters need to be considered to prepare an ideal PLGA-based nanoparticle. The PLGA molecular weight and the ratio of poly-lactic acid to poly-glycolic acid can be manipulated to obtain the desired degradation rate and mechanical strength (Arpagaus, 2019b; Danhier et al., 2012; Pandey & Jain, 2015). The higher molecular weight of PLGA possesses more structural stability, therefore it degrades slowly in *in-vivo* (Arpagaus, 2019b; Essa et al., 2020). To modify the degradation rate of the polymer, different end-capped functional groups also can be used. It has been shown that ester end-capped polymers degrade slower than acid-end capped polymers, thus, useful for slower release applications (Essa et al., 2020). PLA is rigid, and hydrophobic because of methyl groups' existence. Meanwhile, PGA is more flexible and less hydrophobic (Blasi, 2019; Essa et al., 2020). Therefore, PLGA copolymers that contain a high proportion of lactic acid are hydrophobic, making them absorb water slightly, and degrade more gradually than PLGA that contains a high amount of glycolic acid (Blasi, 2019; Pandey & Jain, 2015).

PLGA copolymers that made up of D,L-PLA, and D,L-PGA are amorphous and allow the embedded active ingredients to disperse more homogenously than in the semi-crystalline form of copolymers which comprised of L-PLA and L-PGA) (Blasi, 2019; Hirenkumar & Steven, 2012; Pandey & Jain, 2015). For this amorphous state of PLGA, the important physical-chemical property is the glass transition temperature (*Tg*). PLGA polymer at a rubbery state is vulnerable to chemical and physical changes which in turn could influence the release mechanisms and kinetics of the embedded active ingredients. The *Tg* decreases in conditions such as 1) low lactic acid content, 2) low PLGA molecular weight, and 3) addition of compounds such as excipients or active ingredients (Blasi, 2019). An amorphous form of PLGA co-polymers that consists of 70% lactic acid content is believed to be suitable for drug delivery application (Essa et al., 2020).

The surface of PLGA nanocarriers can be modulated to prevent it from being recognised and phagocytosed by the pulmonary macrophages. Polyethylene glycol (PEG) and chitosan (natural polymer) are commonly used copolymer coated onto the surface of the PLGA carrier to shields it from being taken up by opsonins (an antibody that makes foreign particles susceptible to phagocytosis) and promotes stronger cellular interaction and retention (Danhier et al., 2012). The addition of surfactant in the polymeric solution (polyvinyl alcohol (PVA), polysorbate 80, 60, and 20, poloxamer or poloxamine) is suggested to prevent particle aggregation and optimise the colloidal stability by increasing miscibility and dispersion of drugs in the polymeric solution (Beck-Broichsitter et al., 2012; Essa et al., 2020). Besides, a combination of PLGA-based nanoparticles with ligands that specifically bind to receptors on the cell of interest, enables the PLGA carrier to enter the cell by receptor-mediated endocytosis (Essa et al., 2020). This strategy could enhance the targeted delivery systems of the PLGA nanoparticles to the targeted site of action.

For the spray parameters, the preferred organic solvents used in the nanospray drying of PLGA polymers are dichloromethane (DCM), acetone, acetonitrile, ethyl acetate, and mixtures of DCM/methanol. The boiling point of DCM and acetone which is 40°C and 56°C respectively has allowed a low drying temperature which results in a fast-drying process and hampers particles from binding to or agglomerating on the walls. The ideal inlet drying gas temperature for PLGA dissolved in DCM lies between 29° and 32°C (Arpagaus, 2018, 2019b; Schafroth et al., 2012), meanwhile, the outlet temperature is between 21°C and 35° C, which is below the glass transition temperature of most PLGA polymers (about between 37° C and 54°C) (Arpagaus, 2019a; Hirenkumar & Steven, 2012). The outlet temperature must not exceed the PLGA biopolymer glass transition temperature to avoid softening and yield reduction (Arpagaus, 2019b). Therefore, heat-sensitive pharmaceuticals are the most suitable materials to be used in nanospray drying (Amsalem et al., 2017; Arpagaus, 2019a).

The slow and gentle drying produces an almost smooth and spherical surface of compact carrier particles (Ahmad et al., 2015; Arpagaus & Meuri, 2010). However, the smooth surface of the carrier particles may result in the generation of auto-adhesive layers surrounding the carrier particles. Consequently, the micronised drug particles could agglomerate thus hinders their detachment during inhalation. To improve the aggregation and disaggregation of the micronised powder particles, chitosan may be used as a co-polymer as it gives a rougher surface (groove space) onto the carrier particle which is a good space to bind a drug and help it spread in the oral cavities (better aerosolisation properties) (Ahmad et al., 2015).

A diluted polymer solution with solids concentration ranging from 0.1 to 1% (w/v) could produce spray-dried particles down to a size of 100 nm (Arpagaus, 2018; Arpagaus et al., 2018). Feed rates depend on the spray cap membrane's size, type of formulation, temperature inlet and spray rate. The feed rate must be adjusted to within a range of 10mL/h to 16mL/h when using a 4.0 uM spray cap (Arpagaus, 2012). Typically, Inert gases such as nitrogen and carbon dioxide are used as drying gases to avoid a blast of explosives from occurring. The concentration is adjusted to be less than 4% and the inert gases are recirculated entirely within the circuit (Arpagaus et al., 2018). The nozzle spray pretreated with a surfactant solution (leucine) could generate a free-flowing powder with a satisfactory aerosol functioning. This could be

beneficial for the nanospray dryer to produce efficient powder for inhalation (Patrizia et al., 2014). The encapsulated product must be kept under a controlled condition and a stabilizer may be added into the feed formulation to maintain its activity after nanospray drying. Because most nanosprays dried powders are amorphous after a brief drying time, it is important to store the powders under dry conditions to prevent recrystallization (Arpagaus, 2019b; Arpagaus et al., 2018; Arpagaus & Meuri, 2010).

Conclusion

Nano spray drying technology is economically simple, easy to use, and very efficient in the formulation of nanospray inhalation. PLGA has several advantages to be used in nanospray inhalation according to its properties such as biodegradable, biosafety, biocompatibility, versatility in formulation and functionalization. The inhaled drugs provide a rapid and direct local effect on the respiratory regions as compared to oral drugs, suggesting its beneficial uses in diseases associated with respiratory disorders. Several formulation parameters (hydrophilicity/hydrophobicity of drug, polymer molecular weight, polymer composition, and degradation rate) and spraying parameters (spray mesh diameter, spray rate, sample concentration, sample flow rate, drying gas temperature, solvent types, selection of excipients, stabilizers, or surfactants) are important to take into considerations as they have a great impact on the particle size, production yield, encapsulation efficiency, solid-state solubility, and controlled release profiles of the engineered nanoparticles. The demand for large scale production of powder particles may increase especially during the pandemic of the severe acute respiratory syndrome (SARS) caused by coronaviruses. Future research should, therefore, focus on a pilot- and industrial-scale commercialization of this technology to promote the use of nanospray inhalation for the treatment of SARS coronaviruses infection.

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Conflict of Interest

The authors declare no conflict of interest.

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Antimicrobial Study of Chloroform Fraction from the Leaves of *Entada spiralis* Ridl.

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ABSTRACT

Introduction: *Entada Spiralis* Ridl., or locally identified as Sintok, contains flavonoid, saponin, tannin, and glycoside, compounds that have antifungal and antibacterial activities. This research aims to identify bioactive compounds and determine the antimicrobial activity from crude and fraction of *E. spiralis* extract.

Methods: The crude extract was prepared by macerating the leaves with chloroform, and then proceeded to fraction it by vacuum liquid chromatography with Dichloromethane (DCM)/Hexane (Hex) (1/9) and Dichloromethane (DCM)/Methanol (MeOH) (9/1) solvent system. Disk Diffusion Test and Microdilution Assay evaluated the extracts' antimicrobial activity against *S. aureus, E. coli* and *C. albicans*. The determination of bioactive compounds was done by Thin Layer Chromatography (TLC). Determination of Total Phenolic (TPC) and Flavonoid Content (TFC) were performed by Folin-Ciocalteu and $AlCl_3$ Colourimetric Assay

Results: The greatest inhibition zone against *C. albicans* was obtained from fraction Chloroform ($CHCl_3$) extract with an inhibition zone of 10.33 mm. DCM/MeOH (9/1) effectively killed *S. aureus and E.coli* with an inhibition zone of 11.67 and 12 mm, respectively. The minimum inhibitory concentration (MIC) of $CHCl_3$ crude extract were 1.563 mg/mL for both *E. coli* and *S. aureus*, and 0.781 mg/mL for *C. albicans.* The TLC revealed the presence of tannins, saponin, glycosides, phenol, flavonoid, triterpenoid, and aromatic compound in $CHCl_3$ crude extract. TPC of DCM/MeOH (9/1), $CHCl_3$, and DCM/Hex (1/9) were 50.56 ± 0.188, 51.913± 0.089, 24.16 ± 0.175 mg GAE/g extract.

Conclusion: In conclusion, *E. spiralis* leaves could be a source of active antifungal and antimicrobial agents used for food preservation by using a semipolar solvent for extraction.

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Introduction

Nowadays, researchers pay more attention to natural antimicrobials, such as extracts from plants, to preserve food and modern medicine. This issue is related to the increase of public concern over chemical and synthetic preservatives. About three-quarters of the world population was estimated to depend mainly on plants and plant extracts for their health care because synthetic products could cause postural hypertension, heart failure, and impotence (Zhang et al., 2013). One of the potential plants is *E. spiralis.* Ridley, (synonym, *Entada scheffleri*), locally known as Sintok, Sea Bean, or Beluru, from Leguminosae. Traditionally, it is used for hair treatment and as a cleaner for some skin disease. This plant is a tropical liana or woody climber that could go up to 25 m long (National Park, 2019), and it is commonly found in Australia and Southeast Asia. A previous study showed that *E. spiralis* stem bark contains flavonoid, saponin, tannin, and glycoside (Harun et al., 2014). These compounds have anti-inflammatory, antifungal, anti-yeast, and antibacterial activities (Arabski et al., 2012). Several researchers already found the potential of genus Entada as an antimicrobial agent. For example, *Entada rheedii* ethanol extract was reported to display moderate inhibitory against *S. aureu*s and *C. albicans* (Ram et al., 2004). On the same species, the extract of methanol and fraction of *Entada spiralis* stem bark showed antifungal activity against *T. mentagrophytes, M. gypeseum, T. mentagrophytes, S. aureus,* and *S. epidermis* (Harun et al., 2014). Meanwhile, the chloroform extract of *Entada spirali*s leaves showed flavonoid, phenol (Mohammad, 2017). According to the previous result related to the potential agent of *E. spiralis* leaves as an antimicrobial agent, we aimed to enlarge the potential of the crude chloroform extract of *E. spiralis* leaves as food preservation against *S. aureus, E. coli,* and *C. albicans*.

Methodology

Maceration

E. spiralis leaves were obtained from Tasik Chini, Pahang, with voucher specimen no. KMS-5228. The 900 grams of dried leaves were later milled into a fine powder and macerated with 4 L of chloroform for 48 h Furthermore, the extracts were then filtered using a Whatman No. 1's filter paper. Chloroform was completely evaporated using a rotary evaporator at 40 °C. Once thoroughly dried, the extract was placed in a 5 L Erlenmeyer and stored in a fridge at a temperature of -4 °C before further analysis.

Phytochemical Screening Test – Thin Layer Chromatography (TLC)

TLC silica gel plate 60 F254 (Merck, Germany) was sized into 5 x 3 cm. The 5 µL of the extract was spotted in the middle bottom of the TLC plate. The DCM/Hex (9/1) and DCM/MeOH (9/1) solvent system solutions were used as mobile phases that were already tested from the preliminary research. The developing stage was conducted in a covered TLC developing chamber. The solvent was evaporated in a fume hood at 29 °C. Some colouring agents and wavelength, such as Vanillin, Dragendroff's, FeCl3, UV 365 nm, UV 254 nm, iodine pearl, and concentrated H2SO4 was given onto the different developed plates to determine several bioactive compounds such as saponin, terpenoid, alkaloid, flavonoid, phenol, tannin, aromatic compound, and conjugated compound by Rf values calculations.

Fractionation of Extract by Vacuum Liquid Chromatography (VLC)

In the present study, a modified method from Mohammad (2017) was performed. It conducted a dilution of 10 grams of crude extract into 25 mL of chloroform solvent. The activated silica gel 60 PF254 (Merck, Germany) through a 24 h heating at 80 °C was dipped into the extract solution. Sample and silica gel were kept agitated in a Hotplate Stirrer (Lab Tech, Korea) at 70 °C until was mixed gently. The silica gel was loaded into the VLC column until the solvent front reached 5 cm of the column height. Hexane was utilized to rinse the column by pressed the layer with Aspirator A-1000S (EYELA, Japan). The silica gel was stabilized by setting it down overnight in the VLC column. Ten (10) grams of the crude extract was submitted into VLC, eluted using a gradient system of DCM/Hex (9/1) and DCM/MeOH (9/1) as much as 1 L for each. The solvent system's use depended on the result of Rf value in preliminary research by using TLC. Furthermore, every 200 mL of the fraction, or based on the UV lamp band result, was separated respectively in Erlenmeyer flask. The fractions were tested with the TLC profile to identify the specific compounds. The extract for each solvent system was combined and evaporated in the rotary evaporator IKA HB10 Basic (Buchi, Switzerland) to dryness at 40 °C.

Disk Diffusion Agar, Kirby-Bauer Method

The antimicrobial activity was conducted for crude (CHCl3) and fractional (DCM/Hex (9/1), and DCM/MeOH (9/1)) extracts using a modified method from Harun et al. (2014) was performed in the present study. A 1600 mg/mL stock solution was formulated by dissolving 1.6 g of sample in 1 ml of the specific solvent system depend on the polarity of the fractions. The serial two-fold dilution was done to make the several concentrations ranged from 12.5 mg/mL - 1600 mg/mL. The 20 ml/µL of

each concentration was dipped onto paper discs (Whatman AA disc, 6 mm, United States of America) by using a micro-pipette. The discs were evaporated at the laminar airflow cabinet (Erla CMP Series, Malaysia) until the solvent was evaporated completely. All discs were stored at -5 °C in Chiller LC-213LD (Law Chain, Taiwan) for further analysis.

The positive control of antifungal, gram-negative bacteria and gram-positive respectively employed 100 µL Nystatin (Oxoid, United Kingdom), 10 µL ampicillin (Oxoid, United Kingdom), and 10 µL Gentamicin (Oxoid, United Kingdom). The Chloroform, DCM/Hex (9/1), and DCM/MeOH (9/1) (Merck, Germany) was used as a negative control. The pure culture of E. coli, S. aureus, and the fungal strain C. albicans were collected from the Basic Medicine Science of International Islamic University of Malaysia. Bacterial and fungi were incubated in 24 h at 37 °C in Mueller Hilton-Agar (Oxoid, United Kingdom) and Saboraud Dextrose Agar (Becton, United States of America). The OD of bacteria was 0.1 at 600 nm UV-Vis spectrophotometer (Secomam, United States of America). Thus, the standard inoculation of bacteria was standardized at 1.5 x 106cell/mL and was swabbed on an agar plate using a sterile cotton bud. The sterile paper discs were aseptically transferred onto the inoculated agar plates' surfaces and were submitted to 24 h of incubation at 37 ℃. The effect of the antimicrobial agent was indicated through a clear zone. The determination of this assay was conducted in triplicate.

Minimum Inhibitory Concentration (MIC), by Broth Microdilution Assay

The present study modified a method by Harun et al. (2014) and Mohamad (2012) by diluting the microbial stock into the Mueller Hilton-Broth (Oxoid, United Kingdom) to an absorbance of 0.11 at 600 nm for bacteria and Saboraud Dextrose Broth (Becton, United States of America) to the absorbance of 0.6 at 450 nm for fungi. The 1 mL inoculum was added to 100 mL of sterile broth and diluted until a reached number of OD 105 cells/mL for fungi and 1.5 x 106 4cells/mL for bacteria. The first row of the 96 well microtiter plate (Trueline, United States of America) was filled with 180 µL of 1/100 diluted microbial solution and 100 µL for the rest. Therefore, the 20 µL of two-fold serial dilution 50 mg/mL - 0.39 mg/mL concentration extract was diluted with DMSO. The micro-plates were incubated at 37 ℃ for 24 h. The 20 uL 0.5% (w/v) of MTT (3-(4,5-dimethylthiazol-2-yl) - 2,5 diphenyl tetrazolium bromide (Life Technologies, United States of America) and Phosphate Buffered Saline solution (Base, Singapore) was put to each well and re-incubation for 2 h for fungi and bacteria. The yellow color indicated the inhibition of microbial growth; meanwhile, the dark blue indicates microorganisms' presence.

Minimum Bactericidal Concentrations (MBC) and Fungicidal Concentrations (MFC)

The MBC and MFC were used for each concentration on the solvent system of all microorganisms tested. The MBC and MFC pursued the lowest concentration by calculating fewer than three colonies to achieve approximately 99% inhibition growth. 100 µL of the mixture from MIC that showed the positive result was incubated on SDA (for fungi) and MHA (for bacteria) at 37 ℃ for 24 h.

Total Phenolic Content (TPC)

The Folin-Ciocalteu assay was performed to identify the amount of TPC in E. spiralis leaves based on Mohamad's method (2012). 50 µL of the extract was formulated from 2 mg/mL with the original solvent. The standard of gallic acid solution (7.8 -1000 µL/mL) was diluted into 100 µL of methanol, where the blank was using methanol. The sample was diluted with a ratio of 4:1 (water: Folin- Ciocalteu phenol reagent) and 50 µL of 1 M Sodium carbonate (Na_2CO_3) solution in water. 25 µL sample or standard combined with 100 µL Folin Ciocalteu phenol reagent were filled into a 96-well plate and incubate for 5 min at 37 ℃. 75 µL Na_2CO_3 was added and was kept from light for 1 h. The result's absorbance was analyzed at 765 nm against blank by a multi-detection microplate reader (Infinite M200 Nanoquant, Switzerland).

Results

Phytochemical Screening of Crude Extract

The phytochemical screening results of E. spiralis crude extract by TLC can be seen in **Table 1**. Crude extract showed several bioactive compounds such as; terpene, steroid, terpenoid, phenol, tannin, saponin, flavonoid, and aromatic compound, as shown in **Figure 1**.

Antimicrobial Activity Assay by Kirby-Bauer Test

The chloroform crude extract strongly inhibited the E. coli with an inhibition zone of 10.67 mm in **Table 2**, followed by C. albican and S. aureus with an inhibition zone of 10.33 and 9.67 mm, respectively. Moreover, the fraction DCM/MeOH (9/1) extract was strongly inhibited the *E. coli* with a 12.00 mm inhibition zone in **Table 3**, followed by *C. albicans* and *S. aureus* with an inhibition zone of 9.00 and 11.67 mm, respectively. Among all microorganisms that were used, *E coli* was found to be the most susceptible, whereas *S. aureus* and *C. albicans* were less susceptible to all extracts.

Table 1: TLC Analysis of *Entada spiralis* Crude Extract

Reagent Sprayer	Spot on TLC plate			
	Total spot	Color	Rf	Compound
Nature	7	Yellow	0.1125	Neoxanthin
		Yellow	0.1625	Violaxanthin
		Dark Yellow	0.2	Lutein
		Blue Green	0.275	Chlorophyll b
		Grey	0.6875	Anthocyanin
		Dark Green	0.75	Pheophytin a
		Yellow	0.9375	Carotene
UV_{254} Light	7	Grey	0.1125	Aromatic compound
		Grey	0.1625	
		Dark Grey	0.2	
		Dark Grey	0.275	
		Dark Grey	0.6875	
		Dark Grey	0.75	
		Dark Grey	0.9375	
Sulphuric Acid	3	Green	0.25	Tertepenes
		Yellow	0.5	Steroid
		Pink	0.6875	Steroid
Iodine	3	Brown	0.25	Aromatic and organic compound
		Brown	0.5	
		Dark Brown	0.6875	
UV_{365} Light	1	Fluoresence	0.6875	Terpenoid
$FeCl_3$	2	Green	0.1125	Phenol
		Green	0.357	Tannin
Vanillin	3	Dark Purple	0.175	Saponin
		Green	0.2625	Furastanol
		Violet	0.5375	Spirostanol
$AlCl_3$	1	Orange	0.6155	Flavonoid

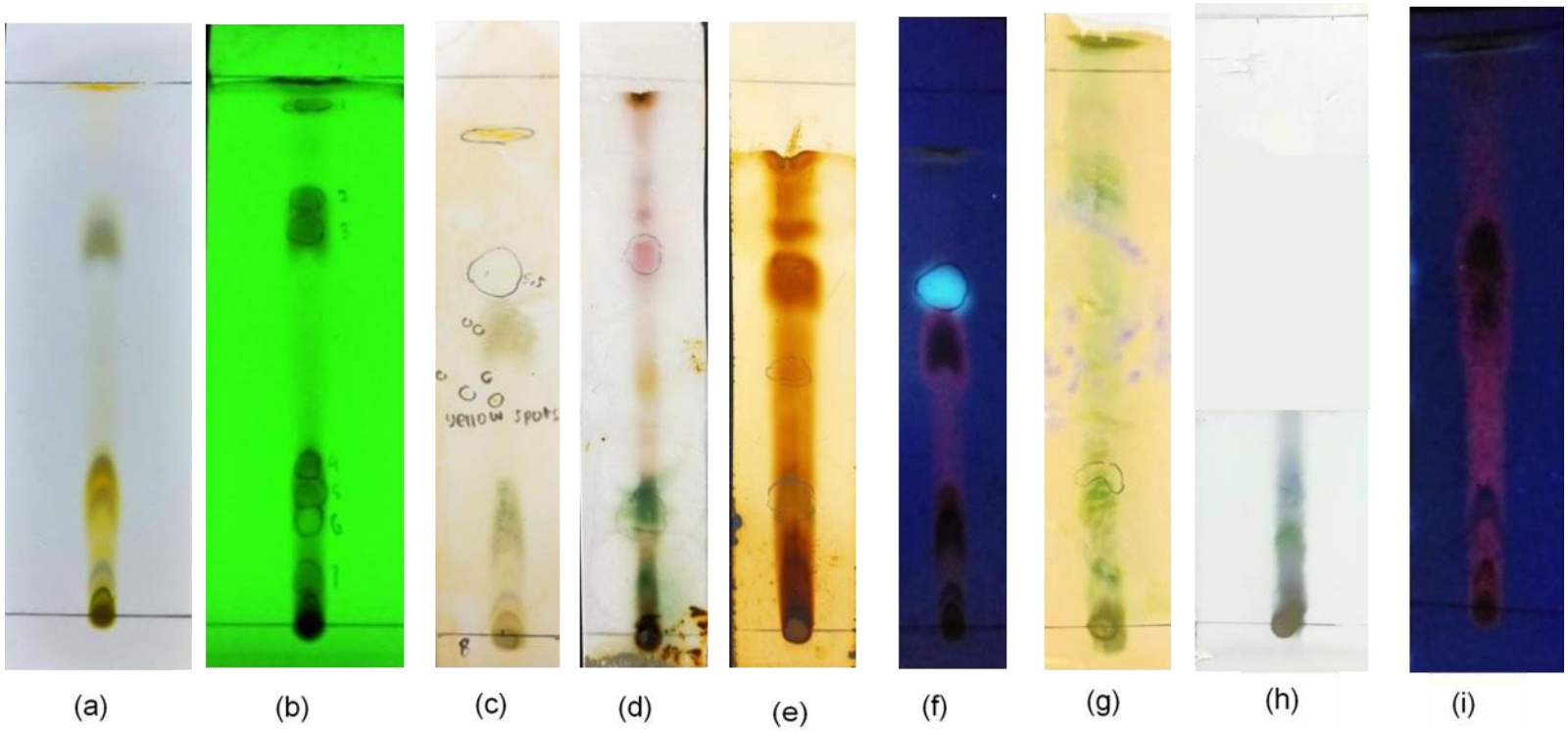

Figure 1: TLC result of *E. spiralis* Leaves Chloroform Extract with Several Conditions; (a) Nature, (b) UV_{254} Light, (c) sprayed by Dragendorff reagent, (d) H_2SO_4, (e) sprayed by Iodine, (F) UV_{365} Light, (g) sprayed by $FeCl_3$, (h) sprayed by vanillin reagent, (I) sprayed by $AlCl_3$

Table 2: *In vitro* Antifungal and Antimicrobial Activity of Fraction and Crude Extract of *E. spiralis* Leaves

Extract	Concentration (mg/mL)	Inhibition zone (mm)[a]		
		EC	SA	CA
DCM/Hex	12.5	-	-	-
	25	-	-	-
	50	-	-	-
	100	-	-	-
	200	6.67 ± 0.577^{c}	-	-
Negative control DCM/Hex		-	-	-
Chloroform	12.5	7.33 ± 1.155^{bc}	7.17 ± 0.288^{b}	
	25	7.67 ± 0.577^{bc}	7.33 ± 0.577^{b}	
	50	7.67 ± 0.577^{bc}	$7.67 \pm 1,443^{ab}$	
	100	8.33 ± 0.577^{abc}	$7.77 \pm 1,328^{ab}$	
	200	9.00 ± 1^{ab}	7.67 ± 0.577^{ab}	
Negative control Chloroform		-	-	-
DCM/MeOH	12.5	8.33 ± 0.577^{abc}	$7.33 \pm 1,527^{b}$	6.33 ± 0.577^{bc}
	25	8.67 ± 0.577^{ab}	$7.67 \pm 0,527^{ab}$	6.67 ± 0.577^{bc}
	50	9.00 ± 1^{ab}	$8.33 \pm 0,577^{ab}$	7.0 ± 0^{b}
	100	9.00 ± 0^{ab}	$9.167 \pm 0,763^{a}$	7.0 ± 0^{b}
	200	9.67 ± 0.577^{a}	9.00 ± 1^{a}	7.0 ± 0^{b}
Negative control DCM/MeOH		-	-	-
Nystatin	100			24.0 ± 1
Ampicillin	10		24.33 ± 1.527	
Gentamicin	10	32.67 ± 0.577		

- No activity; EC; *E. coli*, CA ; *C. albicans*, SA ; *S. aureus;* ±, Standard Deviation (SD); [a] Mean of triplicates. Statistical significance was determined using ANOVA. Differences were analyzed significant ($P<0.05$) on the same microbes. The different notation means significantly different values.

Table 3: *In vitro* Antimicrobial and Antifungal Activity of DCM:MeOH Fraction and Crude Extract of *E. spiralis* Leaves

Extract	Concentration (mg/mL)	Inhibition zone (mm)[a]		
		EC	SA	CA
Chloroform	400	9.33 ± 0.577^{b}	7.67 ± 0.577^{b}	$8.67 \pm 0,577^{ab}$
	800	10.33 ± 1.154^{ab}	$8.833 \pm 1,607^{ab}$	$8.67 \pm 1,155^{ab}$
	1600	10.67 ± 1.154^{ab}	$9.667 \pm 2,081^{ab}$	$10.33 \pm 2,309^{a}$
Negative control Chloroform		-	-	-
DCM / MeOH	400	9.00 ± 1^{b}	8.00 ± 0^{b}	$7.33 \pm 0,577^{b}$
	800	11.33 ± 0.577^{a}	$9.67 \pm 0,577^{ab}$	$8.33 \pm 1,155^{ab}$
	1600	12.00 ± 1^{a}	$11.67 \pm 2,887^{a}$	9.00 ± 0^{ab}
Negative control DCM/MeOH		-	-	-
Nystatin	100			24.0 ± 1
Ampicillin	10		24.33 ± 1.527	
Gentamicin	10	32.67 ± 0.577		

- No activity; EC; *E. coli*, CA; *C. albicans,* SA; *S. aureus;* ±, Standard Deviation (SD); [a] Mean of three replicates Statistical significance was determined using ANNOVA. Differences were analyzed significant ($P<0.05$) on the same microbes. The different notation means significantly different values.

Determination of MIC, MFC and MBC

The MIC results of the positive result fractions against bacteria and fungi are presented in **Table 4**. The Chloroform crude extract showed the MIC value of 1.563 mg/mL against both *E. coli* and *S. aureus,* and 0.781 mg/mL against *C. albicans.* Meanwhile, fraction DCM/MeOH (9/1) extract showed the MIC value of 1.563 mg/mL against *E. coli* and 3.125 mg/mL against both *S. aureus* and *C. albicans.*

According to the result of Minimum Bactericidal Concentration (MBC), the chloroform extract had the lowest MBC result against *E. coli*, *S. aureus*, with 0.39 and 0.781 mg/mL compared to fraction DCM/MeOH (9/1) with MBC results of 0.781 and 1.563 mg/mL and positive control 0.39 mg/mL for Gentamicin (µg/mL) and 0.39 mg/mL for Ampicillin (µg/mL). On the other hand, chloroform extract also gave lower MFC result (0.781 mg/mL) compared to DCM/MeOH (9/1) with an MFC result of 1.563 mg/mL, but still fell behind the positive control Nystatin (µg/mL) with an MFC result of 0.39 mg/mL. In conclusion, the chloroform extract has a promising role as an antimicrobial agent against significant microorganisms, especially bacteria.

Table 4: Minimum Inhibitory Concentration, Minimum Fungicidal (MFC) and Bactericidal Concentration (mg/mL) of *E. spiralis* Crude Extract

Extract	MBC		MFC	MIC		
	EC	SA	CA	EC	SA	CA
Chloroform crude extract	0.390	0.781	0.781	1.563	1.563	0.781
Fraction (DCM /MeOH)	0.781	1.563	1.563	0.781	3.125	3.125
Gentamicin (µg/mL)	0.390	-	-	0.390	-	-
Nystatin (µg/mL)	-	-	0.390	-	-	0.390
Ampicillin (µg/mL)		0.390	-	-	0.390	-

Total Phenolic Content

Total phenolic content of *E. spiralis* leaves extracted by chloroform, fraction DCM/MeOH (9/1), and fraction DCM/Hex (1/9) were 50.56 ± 0.089, 51.913± 0.188, 24.16 ± 0.175 the concentration of phenolic was in terms of equivalent (mg GAE/g of the dry weight of extract), separately.

Discussion

In the Kirby-Bauer method, the restraint zone's size expressed the compound's competences wherein the highest the zone, the more powerful the compound. Higher active compounds from the extracts could cause the higher inhibition zones recognized at s higher concentration of all extracts. Fractionation in several plant extracts brought about improved movement, yet others brought about the loss of the action. For instance, in an extract of fraction DCM/Hex (1/9), the inhibition was only detected at a concentration of 200 mg/mL against E. coli with zone inhibition of 7. Since the CHCl3(100%) and DCM/MeOH (9/1) extract showed promising anti-dermatophytes and antibacterial activity, thus it was chosen to undergo fractionation and was further assayed to investigate the effectiveness of the fractions, except DCM/Hex (1/9). The greatest inhibition zone against C. albicans was obtained from fraction Chloroform (CHCl3) extract with an inhibition zone of 10.33 mm. DCM/MeOH (9/1) effectively killed S. aureus and *E. coli* with an inhibition zone of 11.67 and 12 mm, respectively. Those results were determined by the polarity of the solvent's polarity that may be affected by the bioactive compound after the fractionation process and the chemical nature of its bioactive constituents.

The antifungal and antibacterial potency of several plants was related to secondary metabolites established in some fractionations of the unrefined concentrate, permits the appropriation of bio-active compounds into solvents as per their polarity. The current investigation's perception showed that the antifungal and antibacterial activity of the fraction DCM/MeOH (9/1) and $CHCl_3$(100%) extract of *E.spiralis* leaves maybe showed that the active compound was either modestly nonpolar or polar. This may fill into the natural dissolvable that can be utilized in removing dynamic elements of *E.spiralis* leaves. The phytochemical compound can be isolated. For example, the related movement against contagious and antibacterial action may be associated with the nearness of phytochemicals, such as alkaloid, flavonoid, saponin, sterol, and tannins in the individual portion that previously controlled by TLC profile in past study.

The inhibitions were supported by microdilution assay in which the chloroform crude extract gave the highest MIC value of 1.563 mg/mL against E. coli, 1.563 mg/mL against S. aureus, 0.781 mg/mL against C. albicans. As mentioned before, the polarity of the fraction will affect the compound that, in turn, will affect the efficiency of antimicrobial activity. For an extract of fraction, DCM/Hex (1/9) has nonpolar properties. The nonpolar solution contains only a few bioactive compounds such as; flavonoid, sterol, phenol, alkaloid (Widyawati, 2014), terpenoid (Liu et al., 2011), and diterpenoid (Hidayat, 2014) that dissolved by hexane solvent. Meanwhile, in

semipolar - polar extract of fraction DCM/MeOH (9/1) has more bioactive compounds such as sterol, phenol, flavonoid, and alkaloid (Widyawati, 2014). In Semipolar - polar extract of DCM/MeOH (9/1) may also have polar properties such as alkaloid, phenolic, saponin, carotenoid, tannin, amino acid, and glycoside (Elfirta, 2018).

Terpenoids, tannins, phenolics, and flavonoids are secondary metabolites compounds that naturally exist in several types of plants. The secondary metabolites realized with pharmacological significance are commonly known as bioactive compounds (Swabha, 2018). These bioactive compounds will increase lymphocyte activity, such as macrophage cells, especially compounds such as phenolics and its derivatives. Phenolics play several important functions in plants as an immunomodulator and antioxidant. They give opposition against microbes and predators, other than that phenolics protect cellular membranes and tissues from lipid peroxidation, and fix DNA by electron transfer responses (Shankar et al., 2007).

Flavonoids, particularly catechins, have been known for their antimicrobial activity in Gram-positive and Gram-negative microorganisms (Tsuchiya 2015). Catechins will have collaborate with the cell layer of microbes by authoritative to the lipid bilayer and by inactivating or hindering the union of intracellular and extracellular compounds (Reygaert, 2014). Tannins also fill as guards against microorganisms. Their antimicrobial activity method might be identified with their capacity to idle microbial adhesin, cell envelope transport protein, catalyst several enzymes, that properly known as astringency (Bobbarala, 2012). They are fit for restricting metal particles related to the development of microscopic organisms. In this manner, by consolidating with Ca2+ particles engaged with Gram-negative bacteria's structure, dense tannins influence the bacterial wall cell, disrupting the retention of minor components fundamental for bacterial development (Joseph, 2016).

Conclusion

The CHCl3(100%) extract of E. spiralis uncovered several bioactive compounds such as tannins, saponin, glycosides, phenol, flavonoid, triterpenoid, and aromatic compound through Thin Layer Chromatography (TLC). The highest zone inhibition zone for E. coli was obtained from DCM/MeOH (9/1) extract with a diameter of 12.00 ± 1 mm. The highest inhibition zone for S. aureus was obtained from DCM/MeOH (9/1) extract with a diameter of 11.67 ± 2.887 mm. The greatest inhibition zone for C. albicans was obtained from CHCl3(100%) extract with a diameter of 10.33 ± 2.309 mm. The inhibitions were supported by microdilution assay in which the chloroform crude extract gave the highest MIC value of 1.563 mg/mL against E. coli, 1.563 mg/mL against S. aureus, 0.781 mg/mL against C. albicans. This study suggests that the leaf extract of Entada spiralis has great potential to become a natural preservative for foods, replacing chemical preservatives. A future study is needed to distinguish the isolated specific bioactive compounds that can be done by utilizing an LC-MS or GC-MS examination.

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Conflict of Interest

Authors have no conflicts of interest with this publication.

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Stability of extemporaneous rifampicin prepared with X-temp® oral suspension system.

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ABSTRACT

Introduction: Rifampicin is a first line antituberculosis drug that is commonly used in the treatment of tuberculosis, both in adults and paediatric patients. However, there is a lack of liquid formulation for rifampicin in the market due to the small market size and the physicochemical properties of the drug itself. An innovative new mix called X-Temp® oral suspension system (OSS) has been available in the market as a choice of vehicle for extemporaneous suspension.

Aim: The aim of this study was to prepare rifampicin suspension in the X-Temp® OSS and evaluate its stability following storage at two temperatures – refrigerated (5 °C ± 3 °C) and in a stability chamber (30 °C ± 2 °C/RH 75% ± 5%).

Materials and method: This study investigates the physicochemical and microbiological stability of rifampicin formulated in X-temp® OSS. The rifampicin suspension was prepared at 25mg/ml and kept in two types of amber-coloured storage bottles. The bottles were stored in an open and close storage system at 5 °C (refrigeration) and 30 °C/75% RH (non-refrigerated) and the stability of the product was evaluated at specified time intervals.

Results: It was found that the content of rifampicin remained above 90% of the original concentration throughout the study as required by the standard references. Visual appearance, colour, odour and pH remained unchanged throughout the study period and the extemporaneous preparation was not susceptible to microbial contamination.

Conclusion: Results from this stability study confirmed that the X-temp® OSS is a suitable vehicle for the preparation of extemporaneous rifampicin liquid formulation.

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Introduction

Extemporaneous preparations or compounding is defined as the mixing of ingredients listed in a prescription or drug formula. This term generally refers to a manual process, performed for individual orders and specific patients (Quick *et* al., 1997). Although not being conducted often in the current pharmacy practice, it remains an important area that needs to be taken care of by pharmacists. Extemporaneous products usually need to be prepared for certain patient populations who may have special clinical needs, which could not be met by licensed medicinal products available in the markets. In addition, the fact that certain medications are not available in paediatric-friendly dosage forms also necessitate re-formulation by pharmacists as extemporaneous preparations.

Stability of extemporaneous preparation is an important factor that needs to be considered by formulation pharmacists. Since the preparations are unlicensed, the safety risks are higher (Jackson & Lowey, 2010) and hence it is very important to ensure that the formulation is stable and safe for consumption. Common extemporaneous preparations are usually evaluated to ensure their stability as a way to support the practice.

Rifampicin or also known as rifampin is a first line antituberculosis (TB) drug and is important in the treatment of TB during both the active and continuous phases. It has a very low water solubility (1400 mg/L at 25 °C) with high susceptibility to photodegradation in aqueous form (Yalkowsky & He, 2003). It is also unstable in the presence of heat, air and moisture (Osol, Hoover, & *et* al., 1975).

In clinical settings, rifampicin is commonly available in capsule form whilst oral liquid products for paediatric are rarely produced, which might be related to its instability problems (Glass & Haywood, 2006). Hence, it is a common practice to prepare rifampicin extemporaneously in hospitals, clinics and pharmacies for this specific population. This practice has its challenges to ensure good acceptance and compliance from patients due to several issues such as poor palatability and dispersibility in aqueous solution. To ensure good dispersibility, rifampicin is commonly formulated as suspension with suitable suspending agents and thickeners. One particular study by Nahata *et* al. (1994) described the difficulty to withdraw the prescribed amount of rifampicin from extemporaneous preparations due to problems of powder wetting and dispersion faced by syrup-based formulations. They reported that the presence of a suspending agent is important to ensure accurate dosing each time.

Stability of extemporaneous rifampicin is also being described in the literature. Rifampicin is light-, heat-, air- and moisture-sensitive, hence this may lead to challenges in the storage of its liquid preparations. Readers are directed to other publications that discuss the stability of extemporaneously prepared rifampicin suspensions (Glass & Haywood, 2006; Krukenberg, Mischler, Massad, Moore, & Chandler, 1986; Nahata, Morosco, & Hipple, 1994).

Currently in the clinical settings, rifampicin oral liquid products are commonly prepared by using syrups such as simple syrup and cherry syrup. The mixture of Ora-Sweet® and Ora-Plus® syrups are also being suggested. In recent years, an innovative and new mix called X-Temp® oral suspension system (OSS) has been available in the market as a choice of vehicle for extemporaneous syrup and suspension. It is a complete OSS with suspending agent, stabilizer and is mildly flavoured (orange flavour), with minimal preservation. The availability of this OSS could help to accelerate the preparation process of extemporaneous products and potentially offer good stability and palatability of the prepared product. The presence of suspending agents in X-Temp® has made it a suitable carrier to be used in the extemporaneous preparation of low water-soluble drug powder such as rifampicin.

However, with the multiple problems associated with rifampicin stability, a study on rifampicin in X-Temp® OSS is needed. Hence, this study was designed to ensure that the OSS has the ability to preserve rifampicin efficiently. We decided to conduct the stability study independently to investigate the suitability of X-Temp® as a carrier for rifampicin. The aim of this study was to prepare rifampicin suspension in the X-Temp® OSS and evaluate its stability following storage at two temperatures – refrigerated (5 °C ± 3 °C) and in a stability chamber (30 °C ± 2 °C/RH 75% ± 5%). The product was kept in either a plastic or a glass bottle and parameters including physical characteristics (colour, clarity and odour), pH, microbial presence and available concentration were evaluated at specific time intervals in two systems; open and closed systems. Detailed methods and results are presented in the following sections.

Materials and Methods

Materials and Instruments

Rifampicin capsules 300 mg (Rifasynt®), X-temp® OSS and HDPE plastic bottles were obtained from BioScenergy International Pv. Ltd. Methanol (QRëc®) was of AR grade and was used as received. Nutrient agar (Merck, Darmstadt, Germany) was used for microbial tests. UV/Vis spectrophotometer (Kinesis Hitachi Model U-2800, Leicestershire, UK) was used to evaluate the concentration of rifampicin in the OSS, pH meter (Hanna Precision pH meter Model pH 211, Merck, Darmstadt, Germany) was used to assess the pH value of the preparation. A designated stability chamber was used to

store the preparation.

Preparation of rifampicin OOS

Rifampicin capsules were opened in order to obtain the powder. The powder then was mixed with OSS in a mortar according to the amount and concentration needed. Rifampicin OSS was prepared at a concentration of 25 mg/mL by using 300 mg rifampicin capsules. The method used for mixing these two ingredients is the geometric dilution whereby the rifampicin capsule content was triturated in a portion of syrup, syrup was added bit by bit until it becomes a slurry paste, the slurry paste was retriturated and the remaining syrup was added gradually while mixing. Both open and close systems experiments were conducted in two types of container; glass and plastic bottle.

Open system

Rifampicin OSS were prepared and stored in glass and plastic bottles, each containing 300 mL of the product at a concentration of 25 mg/mL. The bottles were kept at two different temperatures, 5 °C ± 3 °C (refrigerated) and 30 °C ± 2 °C/RH 75% ± 5% (non-refrigerated) (ASEAN Guidelines on Stability Study of Drug Product). Each day, 4 mL (equal to 100 mg of rifampicin) was withdrawn and transferred into an empty bottle, to simulate the patient's behaviour of opening, withdrawing and closing the bottle every day. At a specific time interval (0, 7, 14, 30, 60 days), the samples withdrawn were evaluated for the physical characteristics (colour, clarity and odour), pH value, rifampicin concentration and microbial presence, as explained in the following subsections. All experiments were conducted in triplicates (n=3).

Close system

Rifampicin OSS were prepared and stored in glass and plastic bottles, each containing 50 mL of the product. The bottles were kept at two different temperatures, 5 °C ± 3 °C (refrigerated) and 30 °C ± 2 °C/RH 75% ± 5% (non-refrigerated). At a specific time interval (0, 14, 30, 60, 90 days), three bottles from each group were evaluated for the physical characteristics (colour, clarity and odour), pH value, rifampicin concentration and microbial presence, as explained in the following subsections. All experiments were conducted in triplicates (n=3).

Evaluation of physical characteristics and pH value

The physical appearance of the withdrawn suspension was inspected at each time point and was compared with the characteristics recorded at day 0. The suspensions were then shaken well to ensure homogenous dispersion and the required volume was withdrawn from the bottles. No bubble was retained after shaking. The pH value of the product was measured by using a pH meter at each time point. Any changes were recorded accordingly.

Evaluation of rifampicin concentration

The concentration of rifampicin at each time point was determined through spectrophotometry approach. Briefly, 0.1 mL of rifampicin OSS was diluted in 10 mL of methanol (primary dilution) and subsequently 0.1 mL from the primary dilution was further diluted with 10 mL of methanol (secondary dilution). The absorbance of the secondary dilution was measured at 480 nm and the amount of rifampicin presence in the product was calculated in reference to a standard calibration curve of rifampicin prepared at 5, 10, 15, 20 and 25 µg/ml (R^2= 0.9983).

Evaluation of microbial presence

This test was conducted to determine the presence of *Escherichia coli*, aerobic bacteria, yeast and mould in the product. A culture media was prepared by dissolving 3g of nutrient agar dehydrated powder in 150 mL of distilled water. The suspension was boiled and kept under vigorous stirring. The solubilized agar was then sterilized by autoclaving at 121 °C for 15 minutes. Subsequently, 0.1 mL of the tested product was pipetted into a sterile petri dish and the agar was then poured into the same dish, before being placed in an incubator at 37 °C for 2 days (Europe, 2011).

Results

The experiments were conducted to determine the potential of X-temp® OSS to produce a stable extemporaneous oral liquid product of rifampicin. The U.S Pharmacopeia and European Pharmacopeia standard was used as a reference in determining the level of rifampicin concentration needed to be present in the product throughout storage and the microbial limit that is allowed to be available in such a product. The calibration curve of rifampicin was prepared between 5 to 25 µg/mL and this range is within the linear range of rifampicin as reported in the literature (Tilinca et al., 2017).

Open system

As presented in **Table 1** and **2**, the physical characteristics (of the suspension remained unchanged throughout the study period (60 days) for both glass and plastic bottles. The pH of the suspension was maintained between 4.17 to 4.35 for plastic bottles and 4.17 to 4.33 for glass bottles, showing no obvious pH changes in 60 days. The concentration of rifampicin in the preparation was also successfully maintained between the accepted ranges of 90% to 110%. Microbial growth was also absent. This finding was observed for both storage conditions, showing that the X-Temp® OSS has the ability to preserve and stabilize the rifampicin suspension in 60 days, in an open system. The overall stability data are summarized in **Figure 1**, **Figure 2**, **Table 1** and **Table 2**.

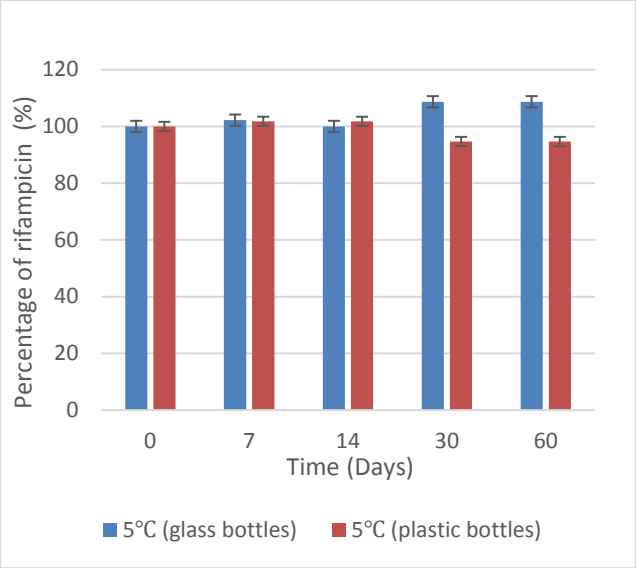

Figure 1: The comparison of rifampicin concentration (%) against time (days) in open system at temperature 5 ºC (n ≥ 3)

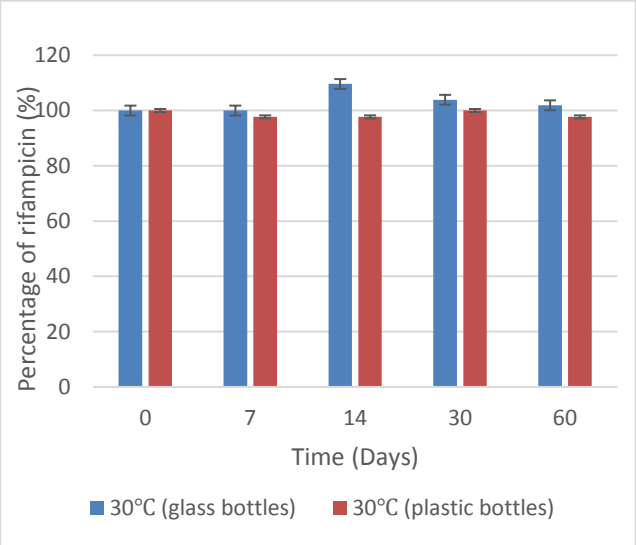

Figure 2: The comparison of rifampicin concentration (%) against time (days) in open system at temperature 30 ºC (n ≥ 3)

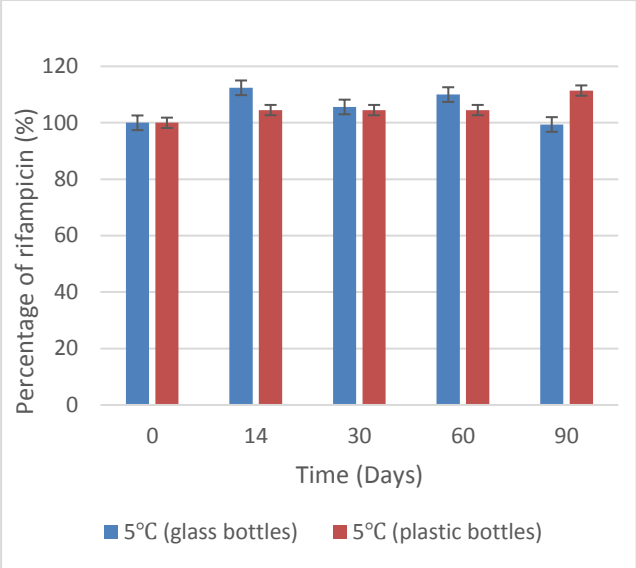

Figure 3: The comparison of rifampicin concentration (%) against time (days) in close system at temperature 5 ºC (n ≥ 3)

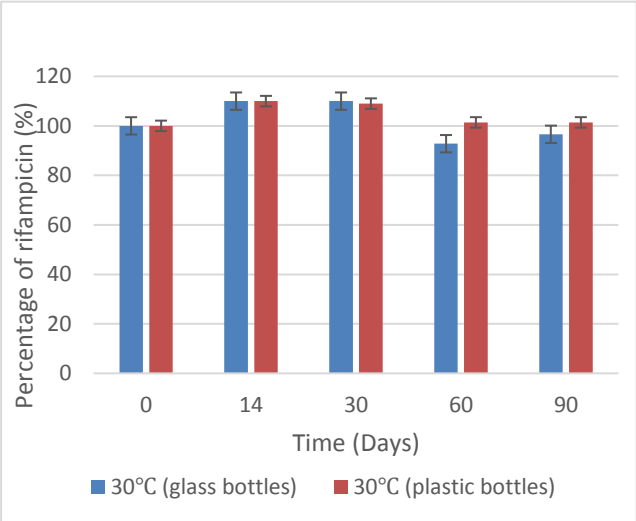

Figure 4: The comparison of rifampicin concentration (%) against time (days) in close system at temperature 30 ºC (n ≥ 3)

Close system

The physical characteristics of rifampicin suspension stored under close system were also maintained throughout the 90 days study period. Fluctuations in terms of concentration could be seen as presented in **Figure 3** and **Figure 4**. The overall characteristics of the rifampicin OSS is presented in **Table 3** and **4**, for plastic and glass bottles respectively.

Table 1: Open system/plastic bottle

Test	Specification	Temperature	Time (Days)				
			0	7	14	30	60
Visual appearance	Colour: Red Clarity: Opaque Odour: Orange	2-8 ºC 30 ºC	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange
pH	4 to 5	2-8 ºC 30 ºC	4.29 4.17	4.29 4.25	4.23 4.31	4.23 4.33	4.37 4.35
Assay	90.0% to 110.0% (USP 2013) ± SEM	2-8 ºC 30 ºC	100.0% ± 0.0 100.0% ± 0.0	101.8% ± 5.6 97.7% ± 5.9	101.8% ± 3.1 97.7% ± 4.7	94.7% ± 4.1 100.0% ± 2.3	94.7% ± 4.1 97.7% ± 0.7
Microbial limit	Aerobic bacteria <1000 cfu/g Yeast & mould <100 cfu/g	2-8 ºC 30 ºC	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms

Table 2: Open system/glass bottle

Test	Specification	Temperature	Time (Days)				
			0	7	14	30	60
Visual appearance	Colour: Red Clarity: Opaque Odour: Orange	2-8 ºC 30 ºC	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange
pH	4 to 5	2-8 ºC 30 ºC	4.31 4.19	4.23 4.25	4.17 4.38	4.17 4.30	4.33 4.38
Assay	90.0% to 110.0% (USP 2013) ± SEM	2-8 ºC 30 ºC	100.0% ± 0.0 100.0% ± 0.0	102.2% ± 6.3 100.0% ± 5.8	100.0% ± 12.6 109.6% ± 3.5	108.7% ± 4.7 103.9% ± 0.6	108.7% ± 4.7 101.9% ± 5.7
Microbial limit	Aerobic bacteria <1000 cfu/g Yeast & mould <100 cfu/g	2-8 ºC 30 ºC	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms

Table 3: Close system/plastic bottle

Test	Specification	Temperature	Time (Days)				
			0	14	30	60	90
Visual appearance	Colour: Red Clarity: Opaque Odour: Orange	2-8 ºC 30 ºC	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange
pH	4 to 5	2-8 ºC 30 ºC	4.17 4.17	4.17 4.65	4.16 4.70	4.16 4.78	4.36 4.79
Assay	90.0% to 110.0% (USP 2013) ± SEM	2-8 ºC 30 ºC	100.0% ± 0.0 100.0% ± 0.0	104.5% ± 8.2 110.0% ± 30.8	104.5% ± 8.2 109.0% ± 0.9	104.5% ± 8.2 101.4% ± 3.9	110.0% ± 8.1 101.4% ± 4.6
Microbial limit	Aerobic bacteria <1000 cfu/g Yeast & mould <100 cfu/g	2-8 ºC 30 ºC	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms

Table 4: Close system/glass bottle

Test	Specification	Temperature	Time (Days)				
			0	14	30	60	90
Visual appearance	Colour: Red Clarity: Opaque Odour: Orange	2-8 ºC 30 ºC	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange	Red, Opaque, Orange Red, Opaque, Orange
pH	4 to 5	2-8 ºC 30 ºC	4.19 4.19	4.23 4.60	4.18 4.71	4.16 4.81	4.36 4.78
Assay	90.0% to 110.0% (USP 2013) ± SEM	2-8 ºC 30 ºC	100.0% ± 0.0 100.0% ± 0.0	109.6% ± 7.1 110.0% ± 1.1	105.6% ± 8.0 110.0% ± 1.5	110.0% ± 7.2 92.8% ± 3.1	99.4% ± 2.5 96.6% ± 2.1
Microbial limit	Aerobic bacteria <1000 cfu/g Yeast & mould <100 cfu/g	2-8 ºC 30 ºC	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms	Conforms Conforms

Discussion

The suspension was prepared from capsules of rifampicin 300 mg. Nahata *et* al. (1994) described four preparation methods that can be used to prepare rifampicin suspension. In this study, we used one of the methods proposed which is triturating rifampicin capsule content in a portion of the carrier, adding more carriers, re-triturating the slurry and adding the remaining carrier while mixing. The authors reported that suspension prepared from capsules could lead to lower-than-expected rifampicin concentration as compared to those prepared from intravenous (IV) preparations (Nahata *et* al., 1994). In this study, we did not compare the stability of the preparations made from IV rifampicin, as the finding obtained from this study showed that the concentration of rifampicin remained above 90% of the original concentration throughout the course of study. The fluctuations in concentration are expected as presented in other previous studies (Baniasadi, Shahsavari, Namdar, & Kobarfard, 2015; Nahata *et* al., 1994). However, the amount of the drug present in the formulation must be ensured to be within the accepted limit (90% – 110%) as outlined by pharmacopoeias.

Based on visual inspection, the rifampicin powder was properly wetted by the OSS and the suspension formed was thick and slurry-like. The presence of three suspending agents in X-Temp® OSS, which are microcrystalline cellulose, carboxymethylcellulose sodium (CMC-Na) and xanthan gum, explains its effectiveness in ensuring the stability of the suspension and prevent caking of rifampicin powder at the bottom of the container. In compounded medications, microcrystalline cellulose is used as an adsorbent, a suspending agent and a capsule diluent (Marques-Marinho, F. D., & Vianna-Soares, C. D. 2013). In addition, microcrystalline cellulose is also considered as a component of the vehicle used for oral suspension (United States Pharmacopeia 2011). Marques-Marinho F. D., & Vianna-Soares also reported in their publication that CMC-Na acts as a capsule disintegrant, a stabilizer, a suspending agent, an emulsifying agent (0.25-1%), a gelling agent (3-6%) and a viscosity-increasing agent (0.1-1%) in compounded medicines. Its application in compounding pharmacies is primarily due to its viscosity-increasing properties. Viscous aqueous solutions are used to suspend powders intended for either topical or oral use (Marques-Marinho, F. D., & Vianna-Soares, C. D. 2013). Xanthan gum is described as anionic microbial polysaccharides, which is believed to have excellent performance in stabilizing suspensions as it will form gel-like network structure by intermolecular interaction (Xue, D., & Sethi, R. 2012).

The X-Temp® OSS could also wet the rifampicin powder well. This ability is important as it relates to the dispersion of the powder and prevents fluctuations in the rifampicin concentrations. Previously reported work on rifampicin suspension by Nahata *et* al., (1994) showed the use of simple syrups was believed to have poor powder wettability, which contributed to a big fluctuation in rifampicin concentration available in the suspension over time, which is risky in the administration of drugs such as rifampicin (Nahata *et* al., 1994).

Dhanapal *et* al. (2012) reported on the formulation and evaluation of rifampicin suspension with single suspending agent. The authors used carboxymethylcellulose sodium, sodium starch glycolate, xanthan gum and carbomer 934, with the presence of flavouring agents and preservatives. It was shown that xanthan gum as a single suspending agent would give the best dispersion characteristics as compared to the others (Dhanapal, Manavalan, Chandar, & Chenthilnathan, 2012). The combination of agents would undoubtedly improve the dispersion criteria, and this seems to be the case with X-temp® OSS.

The rifampicin suspension prepared by using X-temp® OSS did not show any retained bubbles after shaking. There is no tendency of floating ingredients upon standing as compared to what mentioned by Haslam and his colleagues when they used Ora-Sweet®, Ora-Plus® and simple cherry syrup as their suspension vehicle (Haslam, Egodage, Chen, Rajewski, & Stella, 1999). Bubbles will affect the volume of preparation withdrawn; hence the presence of less bubbles is an advantage in liquid pharmaceuticals.

The pH of the rifampicin OSS was aimed at between 4 and 5. This is in accordance with the stability of rifampicin, which has been reported to be at the maximum between the two pH values (Haslam *et* al., 1999). It was also reported elsewhere that acetate buffers influence the degradation of rifampicin at over ten times degradation rate while chloroacetate and phosphate buffers degrade the drugs three times faster (K.C Jindal *et* al., 1995). X-temp® OSS contains citric acid and sodium acid phosphate as buffers. The presence of both ingredients will produce a citrate-phosphate buffer which is also known as McIlvaine buffer. The buffer system used in X-temp® OSS covers a pH range from 2.2 to 8.0. The presence of citric acid also serves as an antioxidant which will prevent the oxidative side reaction of rifampicin. The findings of this study as shown in Table 1, 2, 3 and 4 proved that the pH of rifampicin in X-temp® OSS remained at between pH 4 and 5 throughout the study, which makes the X-temp® OSS a suitable carrier for this drug.

The rifampicin OSS also prepared void of any microbial contamination, despite being prepared at non-sterile room condition. This is an important characteristic of an oral extemporaneous preparation, as the shelf life of such product is usually limited by the growth of microorganisms besides the stability of the active

ingredient itself. The incorporation of preservative(s) is usually needed for extemporaneous products to be safe and stable for a longer period. The preservatives used in this OSS covers a wide range of microorganisms, which is the reason for the absence of microbial contamination throughout the study period. Moreover, the pH of the OSS that maintained between 4 and 5 is also a non-favourable condition for the growth of common microorganisms, which might be an added value in the preservation of the rifampicin OSS (Jin & Kirk, 2018).

Several publications reported on the stability of rifampicin extemporaneous preparation by using different syrups (Allen & Erickson, 1998; Baniasadi *et* al., 2015; Krukenberg *et* al., 1986). The products were kept at either room temperature or under refrigeration. Krukenberg and coworkers showed a four-week stability period for 1% rifampicin suspensions formulated using Syrup NF, two commercially available simple syrup, wild cherry syrup and fruit-flavoured syrup. Allen Jr. L.V. and his team reported a stability period of four weeks for 1% rifampicin suspensions prepared using simple syrup, wild cherry syrup or fruit-flavoured syrup and stored under refrigeration. Baniasadi. S. and his colleagues also reported a stability period of 28 days (4 weeks) for a preparation of 120 mL of 10 mg/mL rifampicin (1%) prepared by using simple syrup and stored under refrigeration. Nahata *et* al. (1994) found that rifampicin was stable in extemporaneous preparation for 56 days (8 weeks) in room temperature while a recent study by Cober *et* al. (2010) said that at least there are 99% of the initial rifampicin throughout the 60 days study period. The formulation was prepared by using rifampicin 20mg/ml (2%) in a mixture of Ora-Plus® with either Ora-Sweet® or Ora-Sweet SF and stored at room temperature (Cober, Johnson, Lee, & Currie, 2010).

Concerning the X-Temp® OSS, the combination of different carefully chosen materials has managed to preserve the rifampicin and stabilize the suspension. The stability was shown to be preserved for 60 days in the open system. The type of bottles was previously described as an important factor in determining the stability of rifampicin suspension (Dhanapal *et* al., 2012). There were also reports on the increasing concentration of rifampicin during storage time, in which the authors believe that this is due to the binding of rifampicin to the plastic bottles and incomplete wetting and dispersion of the rifampicin powder during preparation (Baniasadi *et* al., 2015).

However, Allen and coworkers did not observe this. They reported that the stability of 25mg/mL (2.5%) preparations of rifampicin, extemporaneously prepared in 3 vehicles; 1:1 Ora-Sweet:Ora-Plus, 1:1 Ora-Sweet SF:Ora-Plus, and cherry syrup, stored in polyethylene terephthalate (PET) prescription bottles in the dark was 28 days in both 5 °C and 25 °C (Allen & Erickson, 1998). This could mean that the binding of rifampicin to plastic bottles may happen with certain types of plastics. Hence, care must be taken to choose good plastic bottles such as PET and HDPE for storage to ensure that the concentration of rifampicin remains stable throughout the declared stability period. Another possible explanation would be that the suspension system itself has managed to prevent the adsorption of rifampicin on the bottles. This, however, would necessitate a more detailed study on the adsorption characteristics of the extemporaneous rifampicin suspension on different packaging materials.

Conclusion

The price of syrups used in extemporaneous preparations is an important factor being considered by healthcare institutions. The ability of a carrier system to ensure the stability of the product is also important. X-Temp® OSS can help to maintain the stability of rifampicin extemporaneous preparations for up to 60 days in an open system and up to 90 days in a close system. In such practice, the patients do not need to visit the hospital too often to obtain their drug supply. The pharmacy department can also prepare stocks of rifampicin suspension and keep in a close system for up to 90 days. This is highly convenient and can increase the working efficiency in a busy pharmacy.

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Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article. The authors acknowledge the contribution of BioScenergy International Pv. Ltd. for supplying the X-temp® OSS, rifampicin capsules and plastic HDPE bottles used in this study.

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