

Kaempferia parviflora Rhizome Extract Exhibits Modest Antimicrobial Activity Against UTI Pathogens: A Preliminary Assessment

Evana Kamarudin^{1,3*}, Nurul Fitriyah Mohd Alwi², Razif Dasiman^{2,3}

¹Centre for Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

²Department of Basic Sciences, Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Selangor, Malaysia

³Optometry and Vision Science Research Centre (iROVIS), Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam Campus, 42300, Selangor, Malaysia

ABSTRACT

Background: The global rise in antimicrobial resistance (AMR) presents a critical challenge in treating urinary tract infections (UTIs), which remain one of the most common bacterial infections worldwide. **Methods:** In response to the need for alternative treatments, this study evaluated the antimicrobial activity of an aqueous extract of *Kaempferia parviflora* (Black Ginger) rhizome against three UTI-associated pathogens: *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. **Results:** Phytochemical analysis confirmed the presence of terpenoids, flavonoids, and alkaloids—compounds commonly associated with antimicrobial effects. Despite this, antimicrobial susceptibility testing (AST) showed weak inhibitory activity, with inhibition zones ranging from 8–9 mm. The extract demonstrated high minimum inhibitory concentration (MIC) values, with concentrations up to 1000 mg/mL required to inhibit bacterial growth, and no bactericidal effects were observed in minimum bactericidal concentration (MBC) assays. **Conclusion:** These findings indicate that the aqueous extraction method may not have effectively extracted or preserved the active antimicrobial constituents. Although the extract exhibited limited antibacterial activity, the presence of bioactive compounds suggests potential for further investigation. Future studies should focus on optimizing extraction methods, isolating individual active compounds, and assessing synergistic effects with existing antibiotics. Such approaches may enhance the antimicrobial efficacy of *K. parviflora* and better support its development as part of a plant-based strategy to combat AMR.

Keywords:

Kaempferia parviflora; antimicrobial activity; urinary tract infections; phytochemical analysis; herbal medicine

INTRODUCTION

Urinary tract infections (UTIs) are a significant health concern, affecting millions of people worldwide, and are one of the most common bacterial infections encountered in clinical practice. These infections can affect any part of the urinary system, including the kidneys, bladder, ureters, and urethra, but they most commonly involve the lower urinary tract—particularly the bladder (cystitis) and urethra (urethritis). Women, due to their shorter urethra, are disproportionately affected, with an estimated 50–60% of women experiencing at least one UTI during their lifetime (Kot, 2019). In addition, UTIs are prevalent in hospital settings, where catheterization and other medical procedures often increase the risk of infection, particularly in elderly and immunocompromised patients.

The primary causative agents of UTIs are bacteria, with *Escherichia coli* (UPEC) accounting for up to 80–85% of

cases, especially in uncomplicated infections (Tamadonfar et al., 2019). Other significant pathogens include *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis*, among others. These bacteria often colonize the urinary tract, leading to symptoms such as frequent urination, dysuria (painful urination), and abdominal pain. If left untreated, UTIs can escalate into more serious conditions such as pyelonephritis (kidney infection), which can cause permanent kidney damage and septicemia.

Although antibiotics are the standard treatment for UTIs, the rise of antimicrobial resistance (AMR) poses a growing challenge. Overuse and misuse of antibiotics, both in healthcare and agriculture, have accelerated the development of resistant strains of bacteria. Multidrug-resistant (MDR) bacteria, such as *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, are becoming increasingly common and difficult to treat (Uddin et al., 2021). The emergence of these resistant strains has rendered some commonly used antibiotics ineffective, leading to longer hospital stays, increased healthcare costs, and higher mortality rates.

* Corresponding author.

E-mail address: evana@uitm.edu.my

Journal homepage: <https://journals.iium.edu.my/ijahs/index.php/IJAHS>

It is estimated that, by 2050, antimicrobial resistance could result in 10 million deaths annually if new antimicrobial agents are not developed (Walsh et al., 2023). Given the global urgency surrounding antimicrobial resistance (AMR), the search for alternative antimicrobial therapies is becoming increasingly critical. Natural products, especially those derived from medicinal plants, have been used for centuries in traditional medicine and are now being actively explored as sources of novel antimicrobial agents. *Kaempferia parviflora* (Black Ginger), native to Southeast Asia and traditionally valued for its anti-inflammatory, antioxidant, and aphrodisiac effects, has emerged as a plant of pharmacological interest. Its rhizome contains a diverse range of bioactive compounds—such as flavonoids, terpenoids, and alkaloids—that have demonstrated antimicrobial, anticancer, and anti-diabetic activities in previous studies (Lee et al., 2016; Chen et al., 2018). In the context of AMR, these properties are particularly relevant, as they may offer natural alternatives or adjuncts to synthetic antibiotics. While studies have reported antibacterial activity of *K. parviflora* extracts against organisms such as *Staphylococcus aureus* and *Escherichia coli*, limited research has been conducted on its efficacy against urinary tract infection (UTI)-causing and multidrug-resistant pathogens like *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Investigating its potential against these clinically significant bacteria could contribute to the development of new therapeutic strategies for UTIs and support broader efforts to address the AMR crisis. This study aims to investigate the antimicrobial activity of *K. parviflora* rhizome aqueous extract against three key UTI pathogens: *E. coli*, *K. pneumoniae*, and *P. aeruginosa*. Specifically, we seek to determine whether the extract can inhibit the growth of these pathogens and explore its potential as a natural antimicrobial agent. By conducting phytochemical analysis and evaluating the extract's performance in Antibiotic Susceptibility Testing (AST), Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays, this study provides a comprehensive assessment of *K. parviflora*'s antimicrobial properties. Given the global rise of antimicrobial resistance, identifying plant-based alternatives to traditional antibiotics is essential for future infection control strategies.

MATERIALS AND METHODS

Sample Collection and Preparation

The rhizome of *Kaempferia parviflora* was harvested from Rumah Hijau, located at UiTM Cawangan Selangor, Puncak Alam. The rhizomes were washed thoroughly under running water to remove soil and other impurities. Once cleaned, the rhizomes were cut into thin slices to facilitate drying. The sliced rhizomes were dried in an oven at 40°C for three days. Drying at a relatively low temperature

ensures the retention of heat-sensitive bioactive compounds, which could otherwise be degraded at higher temperatures. After drying, the rhizomes were ground into a fine powder using an electric grinder.

The powdered rhizome was then used to prepare an aqueous extract. In this process, 10 g of the rhizome powder was soaked in 100 ml of distilled water (10:1 w/v ratio) and placed in an orbital shaker at 100 rpm for 24 hours. The shaking process ensured proper mixing and facilitated the release of bioactive compounds from the plant material into the water. The mixture was then filtered using Whatman No. 1 filter paper to remove solid residues, resulting in a clear filtrate. The aqueous extract was subsequently freeze-dried for 72 hours using a freeze-dryer to obtain a powdered form of the extract. The freeze-dried extract was stored at 4°C until further use to preserve its bioactive properties.

Phytochemical Analysis

The qualitative phytochemical analysis was carried out to detect the presence of various bioactive compounds, including tannins, flavonoids, terpenoids, saponins, phenolic compounds, and alkaloids in the aqueous extract of *K. parviflora*. Standard methods were employed for the analysis, as described by (Kong et al., 2019). The tests were performed as follows:

Tannins and Phenolics

Ferric chloride (5%) was added to 2 ml of the extract. A blue-black coloration indicated the presence of tannins, while a dark green coloration suggested the presence of phenolic compounds.

Flavonoids

A few drops of 1N sodium hydroxide were added to 2 ml of the extract. The appearance of a yellow coloration confirmed the presence of flavonoids.

Terpenoids

To 2 ml of the extract, chloroform and concentrated sulfuric acid were added. A reddish-brown color indicated the presence of terpenoids.

Saponins

The extract was vigorously shaken with distilled water, and the formation of stable foam indicated the presence of saponins.

Alkaloids

Mayer's reagent (potassium mercuric iodide) was added to the extract, and the formation of a white precipitate

confirmed the presence of alkaloids.

Bacterial Strains and Culture Conditions

The bacterial strains used in this study were *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 10145). These strains were obtained from the Microbiology Laboratory at the Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, UiTM Puncak Alam. The bacterial strains were cultured on blood agar plates and incubated overnight at 37°C to ensure optimal growth prior to testing. Bacterial suspensions were prepared by adjusting the turbidity to 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml) using sterile saline.

Antibiotic Susceptibility Test (AST)

The antimicrobial activity of *K. parviflora* aqueous extract was assessed using the agar well diffusion method. Mueller-Hinton agar (MHA) plates were prepared by pouring 25 ml of the medium into Petri dishes and allowing them to solidify. Bacterial lawns were created by spreading 100 µl of the bacterial suspension evenly across the surface of the agar using a sterile cotton swab. Wells of 6 mm diameter were punched into the agar using a sterile cork borer.

The test was performed in triplicate, with each well receiving 50 µl of the *K. parviflora* aqueous extract at a concentration of 1000 mg/ml. As positive controls, antibiotic discs containing gentamicin (30 µg) and ciprofloxacin (5 µg) were placed on the agar plates for *E. coli* and *K. pneumoniae*, respectively. A well containing 50 µl of distilled water served as a negative control. Plates were incubated at 37°C for 24 hours, and the zones of inhibition (indicating antimicrobial activity) were measured in millimeters using a digital caliper.

Minimum Inhibitory Concentration (MIC)

The MIC was determined using the microbroth dilution method, which is a quantitative test to determine the lowest concentration of the extract that inhibits visible bacterial growth. Serial two-fold dilutions of the extract were prepared in Mueller-Hinton broth (MHB) in 96-well microtiter plates, with concentrations ranging from 1000 mg/ml to 1.95 mg/ml. Each well was inoculated with 10 µl of the bacterial suspension (1.5×10^8 CFU/ml), and the plates were incubated at 37°C for 24 hours. After incubation, bacterial growth was assessed visually by comparing the turbidity of the wells. Clear wells indicated inhibition of bacterial growth, while turbid wells suggested bacterial proliferation. The MIC was defined as the lowest concentration of the extract that prevented visible growth.

All assays were performed in triplicate.

Minimum Bactericidal Concentration (MBC)

To determine the bactericidal effect of the extract, the contents of wells from the MIC assay that showed no visible growth were subcultured onto fresh Mueller-Hinton agar plates. A loopful of the suspension from each clear well was streaked onto the agar surface, and the plates were incubated at 37°C for 24 hours. The MBC was defined as the lowest concentration of the extract that resulted in no bacterial growth on the agar plates, indicating bactericidal activity.

RESULTS

Phytochemical Composition of *K. parviflora* Rhizome Aqueous Extract

The phytochemical analysis of the aqueous extract of *K. parviflora* revealed the presence of three major classes of bioactive compounds: terpenoids, flavonoids, and alkaloids. These compounds are known for their potential antimicrobial properties and their ability to inhibit bacterial growth through various mechanisms, such as disrupting cell membranes and inhibiting metabolic processes. However, tannins, phenolic compounds, and saponins were not detected in the extract (Table 1).

Table 1: Phytochemical Constituents of *K. parviflora* Rhizome Aqueous Extract
Phytochemical Compound Presence (+/-)

Phytochemical Compound	Presence (+/-)
Tannins	-
Phenolic Compounds	-
Terpenoids	+
Flavonoids	+
Saponins	-
Alkaloids	+

The presence of these bioactive compounds suggests that the rhizome of *K. parviflora* has potential antimicrobial properties, although their effectiveness in inhibiting bacterial growth requires further investigation.

Antibiotic Susceptibility Test (AST)

The results of the agar well diffusion method are summarized in Table 2. The *K. parviflora* aqueous extract exhibited weak antibacterial activity against the three tested UTI pathogens. The average inhibition zone for *E. coli* was 8.33 ± 0.58 mm, for *K. pneumoniae* it was $8.67 \pm$

0.58 mm, and for *P. aeruginosa* it was 8.67 ± 0.58 mm. These inhibition zones were significantly smaller than those produced by the positive control antibiotics, gentamicin and ciprofloxacin, which had zones of 24.00 ± 3.46 mm and 41.00 ± 4.00 mm, respectively.

Table 2: Zone of Inhibition (mm) of *K. parviflora* Aqueous Extract Against UTI Pathogens

Organism	Zone of Inhibition (mm) \pm SD	Positive Control (mm) \pm SD
<i>E. coli</i> (ATCC 25922)	8.33 ± 0.58	24.00 ± 3.46 (Gentamicin)
<i>K. pneumoniae</i> (ATCC 13883)	8.67 ± 0.58	41.00 ± 4.00 (Ciprofloxacin)
<i>P. aeruginosa</i> (ATCC 10145)	8.67 ± 0.58	23.67 ± 1.15 (Gentamicin)

While the extract demonstrated some degree of antibacterial activity, the zones of inhibition were small, suggesting limited efficacy compared to conventional antibiotics. This may be due to the use of an aqueous solvent, which could affect the extraction of lipophilic bioactive compounds.

Minimum Inhibitory Concentration (MIC)

The MIC results indicated that the aqueous extract of *K. parviflora* had limited inhibitory activity at lower concentrations. For all three bacterial strains, the MIC was found to be 1000 mg/ml, which suggests that lower concentrations were insufficient to inhibit bacterial growth. Wells containing concentrations below 1000 mg/ml remained turbid, indicating bacterial proliferation (Table 3).

Table 3: MIC of *K. parviflora* Aqueous Extract Against UTI Pathogens

Organism	MIC (mg/ml)
<i>E. coli</i> (ATCC 25922)	1000
<i>K. pneumoniae</i> (ATCC 13883)	1000
<i>P. aeruginosa</i> (ATCC 10145)	1000

These high MIC values further support the observation that the extract has limited antimicrobial efficacy, particularly at lower concentrations.

Minimum Bactericidal Concentration (MBC)

The MBC results revealed that the aqueous extract did not exhibit bactericidal activity at the tested concentrations.

Bacterial growth was observed in all subcultured plates, even at the highest concentration of 1000 mg/ml, indicating that the extract had no bactericidal effect on any of the tested pathogens.

Table 3: MIC of *K. parviflora* Aqueous Extract Against UTI Pathogens

Organism	MBC (mg/ml)
<i>E. coli</i> (ATCC 25922)	No bactericidal effect
<i>K. pneumoniae</i> (ATCC 13883)	No bactericidal effect
<i>P. aeruginosa</i> (ATCC 10145)	No bactericidal effect

The lack of bactericidal activity, coupled with high MIC values, indicates that while the extract can inhibit bacterial growth at higher concentrations, it does not possess the ability to kill the bacteria outright. This suggests that *K. parviflora* aqueous extract may have bacteriostatic, rather than bactericidal, effects.

DISCUSSION

The findings of this study demonstrate that *Kaempferia parviflora* rhizome aqueous extract possesses limited antimicrobial activity against the three UTI pathogens tested: *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Pseudomonas aeruginosa* (*P. aeruginosa*). Although phytochemical analysis confirmed the presence of bioactive compounds such as flavonoids, terpenoids, and alkaloids, which are known for their potential antimicrobial properties, the efficacy of the aqueous extract was lower than expected. The results from the Antibiotic Susceptibility Test (AST), Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) assays provide important insights into the extract's effectiveness.

The phytochemical screening revealed that the rhizome of *K. parviflora* contains terpenoids, flavonoids, and alkaloids, compounds that have been extensively documented for their antimicrobial properties. Terpenoids, for instance, can disrupt bacterial membranes by interacting with the phospholipid bilayer, leading to cell leakage and death (Huang et al., 2022). Flavonoids have been reported to inhibit bacterial growth by damaging cell membranes, inhibiting nucleic acid synthesis, and disrupting bacterial quorum sensing (Shamsudin et al., 2022). Alkaloids, on the other hand, can interfere with bacterial enzyme function and nucleic acid metabolism, further hindering bacterial growth (Chen et al., 2018).

Despite the presence of these bioactive compounds, the antibacterial activity observed in this study was minimal. The aqueous extract demonstrated weak inhibition zones in the AST (ranging from 8-9 mm), and the MIC values were relatively high (1000 mg/ml for all pathogens tested). These results indicate that the extract's antimicrobial efficacy is lower than that of standard antibiotics such as gentamicin and ciprofloxacin, which produced significantly larger inhibition zones.

One potential explanation for the limited antimicrobial activity observed in this study is the extraction method used. Aqueous extraction, while commonly employed for its simplicity and safety, may not be the most effective method for isolating bioactive compounds from *K. parviflora* rhizomes. Water as a solvent is generally less efficient than organic solvents like ethanol or methanol at extracting lipophilic compounds such as terpenoids and flavonoids (Balouiri et al., 2016). Previous studies have shown that ethanol and methanol extracts of *K. parviflora* exhibit stronger antimicrobial activity than aqueous extracts, likely due to the higher solubility of bioactive compounds in organic solvents (Khaing et al., 2020). Thus, future studies should consider using alternative solvents to enhance the yield and potency of the antimicrobial compounds present in *K. parviflora*.

Although the aqueous extract of *K. parviflora* demonstrated limited antimicrobial activity in this study, it is possible that the extract could be more effective when used in combination with other antimicrobial agents. Synergistic interactions between plant extracts and conventional antibiotics have been documented in several studies, where the combination of natural products and antibiotics enhanced bacterial inhibition and reduced the risk of developing resistance (Chen et al., 2018). For example, combining *K. parviflora* with antibiotics such as gentamicin or ciprofloxacin could potentially lower the effective dose of the antibiotic, thereby reducing the likelihood of AMR while maintaining therapeutic efficacy. Future research should investigate the potential synergistic effects of *K. parviflora* in combination with conventional antibiotics.

The increasing prevalence of antimicrobial-resistant pathogens, particularly multidrug-resistant strains of *K. pneumoniae* and *P. aeruginosa*, underscores the importance of identifying new therapeutic agents. While the results of this study indicate that *K. parviflora* is not highly effective as a standalone antimicrobial agent, its

potential as part of a broader antimicrobial strategy cannot be discounted. In the context of AMR, even natural compounds with moderate antimicrobial activity can play a role in reducing the overall use of synthetic antibiotics and mitigating the selection pressure that drives resistance.

The increasing prevalence of antimicrobial-resistant pathogens, particularly multidrug-resistant strains of *K. pneumoniae* and *P. aeruginosa*, underscores the importance of identifying new therapeutic agents. While the results of this study indicate that *K. parviflora* is not highly effective as a standalone antimicrobial agent, its potential as part of a broader antimicrobial strategy cannot be discounted. In the context of AMR, even natural compounds with moderate antimicrobial activity can play a role in reducing the overall use of synthetic antibiotics and mitigating the selection pressure that drives resistance.

Moreover, *K. parviflora*'s antimicrobial properties may be more pronounced against other pathogens not included in this study, particularly gram-positive bacteria. Previous studies have shown that *K. parviflora* extracts can be more effective against pathogens such as *Staphylococcus aureus* (Wungsintaweekul et al., 2010). Expanding the range of tested microorganisms in future research could provide a more comprehensive understanding of the extract's antimicrobial spectrum.

There are several limitations to this study that should be addressed in future research. First, the concentration of the extract tested (1000 mg/ml) may not be optimal for determining its true antimicrobial potential. Testing higher concentrations of the extract, as well as using alternative extraction methods, could yield more robust antimicrobial activity. Second, while this study focused on three UTI pathogens, future research should explore the efficacy of *K. parviflora* against a wider range of microorganisms, including gram-positive bacteria and fungal pathogens.

Additionally, investigating the molecular mechanisms by which *K. parviflora* exerts its antimicrobial effects could provide valuable insights into how it can be harnessed as part of a multi-targeted approach to combat AMR. Studies involving *in vivo* models of infection, coupled with toxicity assessments, would also be necessary to evaluate the safety and efficacy of *K. parviflora* as a therapeutic agent in clinical settings.

CONCLUSION

This study investigated the antimicrobial activity of *Kaempferia parviflora* rhizome aqueous extract against common UTI-causing pathogens. While phytochemical analysis revealed the presence of compounds typically associated with antimicrobial properties—such as terpenoids, flavonoids, and alkaloids—the extract exhibited only weak antibacterial activity. Inhibition zones were minimal, and high MIC values (up to 1000 mg/mL) were required to inhibit bacterial growth, with no bactericidal effects observed.

These results highlight the limited effectiveness of the aqueous extract in its current form and suggest that the extraction method may not have efficiently isolated or concentrated the active constituents. Nevertheless, the presence of these bioactive compounds supports further investigation, particularly into alternative extraction techniques, purification of specific active components, and evaluation of synergistic interactions with conventional antibiotics.

Future work should focus on enhancing the extract's potency through method optimization or combination therapy approaches. Such studies are essential to more accurately assess the therapeutic potential of *K. parviflora* as part of a broader natural antimicrobial strategy in the fight against antimicrobial resistance.

ACKNOWLEDGEMENT

Sincere appreciation to Faculty of Health Sciences, University Technology MARA (UiTM), Selangor Campus, Puncak Alam for the funding which is the generous financial support of this research project and all colleagues for their commitment throughout the research.

REFERENCES

- Álvarez-Martínez, F. J., Barraji3n-Catal3n, E., & Micol, V. (2020). Tackling Antibiotic Resistance with Compounds of Natural Origin: A Comprehensive Review. *Biomedicines*, 8(10), 405–405. <https://doi.org/10.3390/biomedicines8100405>
- Balouiri, M., Sadiki, M., & Ibsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis/Journal of Pharmaceutical Analysis*, 6(2), 71–79. <https://doi.org/10.1016/j.jpha.2015.11.005>
- Chen, D., Li, H., Li, W., Feng, S., & Deng, D. (2018). *Kaempferia parviflora* and Its Methoxyflavones: Chemistry and Biological Activities. *Evidence-Based Complementary and Alternative Medicine*, 2018, 1–15. <https://doi.org/10.1155/2018/4057456>
- Huang, L., Huang, C., Yan, Y., Sun, L., & Li, H. (2022). Urinary Tract Infection Etiological Profiles and Antibiotic Resistance Patterns Varied Among Different Age Categories: A Retrospective Study From a Tertiary General Hospital During a 12-Year Period. *Frontiers in Microbiology*, 12. <https://doi.org/10.3389/fmicb.2021.813145>
- Huang, W., Wang, Y., Tian, W., Cui, X., Tu, P., Li, J., Shi, S., & Liu, X. (2022). Biosynthesis Investigations of Terpenoid, Alkaloid, and Flavonoid Antimicrobial Agents Derived from Medicinal Plants. *Antibiotics*, 11(10), 1380–1380. <https://doi.org/10.3390/antibiotics11101380>
- Khaing, Z. T., San Aung, Y. M., & Aung, Y. M. (2020). A Comparative Study of Phytochemical Constituents and Antioxidant Activities of Rhizomes of *Curcuma aeruginosa Roxb* and *Kaempferia parviflora* Wall. In 3rd Myanmar Korea Conference Research Journal (Vol. 3, pp. 2059-2067).
- Kong, H. S., Musa, K. H., Mohd Kasim, Z., & Abdullah Sani, N. (2019). Qualitative and quantitative phytochemical analysis and antioxidant properties of leaves and stems of *clinacanthus nutans* (Burm. f.) lindau from two herbal farms of Negeri Sembilan, Malaysia. *ASM Science Journal*, 12, 1–13. <https://doi.org/10.32802/asmscj.2019.87>
- Kot, B. (2019). Antibiotic Resistance Among Uropathogenic *Escherichia coli*. *Polish Journal of Microbiology*, 68(4), 403–415. <https://doi.org/10.33073/pjm-2019-048>
- Lee, T., Labrooy, C., Abdullah, T., Ashikin, N., Abdullah, P., & Stanslas, J. (2016). Pre-soak Technique Using Bap or Ethephon to Break Dormancy in Black Galingale (*Kaempferia parviflora*). *J. Agric. & Environ. Sci*, 16(9), 1577–1582. <https://doi.org/10.5829/idosi.ajeaes.2016.1577.1582>
- Shamsudin, N. F., Ahmed, Q. U., Mahmood, S., Shah, S. a. A., Khatib, A., Mukhtar, S., Alsharif, M. A., Parveen, H., & Zakaria, Z. A. (2022). Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative interpretation. *Molecules/Molecules Online/Molecules Annual*, 27(4), 1149. <https://doi.org/10.3390/molecules27041149>

Tamadonfar, K. O., Omattage, N. S., Spaulding, C. N., & Hultgren, S. J. (2019). Reaching the End of the Line: Urinary Tract Infections. *Microbiology Spectrum*, 7(3). <https://doi.org/10.1128/microbiolspec.bai-0014-2019>

Uddin, T, M., Chakraborty, A, J., Khusro, A., Matin, R., Mitra, S., Emran, T, B., Dhama, K., Ripon, K, H., Gajdács, M., Muhammad, Hossain, J., & Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *Journal of Infection and Public Health*, 14(12), 1750–1766. <https://doi.org/10.1016/j.jiph.2021.10.020>

Walsh, T. R., Gales, A. C., Laxminarayan, R., & Dodd, P. C. (2023). Antimicrobial Resistance: Addressing a Global Threat to Humanity. *PLoS Medicine*, 20(7), e1004264–e1004264. <https://doi.org/10.1371/journal.pmed.1004264>

Wungsintaweekul, J., Sithithaworn, W., Putalun, W., Pfeifhoffer, H., & Brantner, A. (2010). Antimicrobial, antioxidant activities and chemical composition of selected Thai spices. / *Songklanakarin J. Sci. Technol*, 32(6), 589–598. <https://www.thaiscience.info/Journals/Article/SONG/10691288.pdf>

Characterisation of *Staphylococcus aureus* Carriage Patterns and Associated Risk Factors Among Pre-clinical and Clinical Students at a Malaysian University

Iman Abdul Aziz¹, Rufaida Muhammad¹, Nur Zarith Fatimah Johari¹, Nur Anisah Noor Habibullah¹, Ain Syakirah Mat Zanggi¹, Niazlin Mohd Taib², Mohd Fahmi Mastuki^{1,3}.

¹Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

²Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.

³Research and Education in Biomedical, Epidemiology and Immunology Aliances (REBEIA), FSK, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Nasal carriage of *Staphylococcus aureus* among healthcare students constitutes a potential reservoir for nosocomial transmission and may predispose carriers to subsequent infections. However, limited data are available regarding carriage patterns and associated risk factors among Medical Laboratory Technology (MLT) students in Malaysia, who are at occupational risk of exposure to pathogens during their training. This study aimed to determine the prevalence of *S. aureus* nasal carriage, characterise carriage patterns, and identify associated risk factors among pre-clinical and clinical MLT students at a Malaysian university. A prospective cross-sectional study was conducted involving 144 MLT students from Universiti Teknologi MARA (UiTM), Puncak Alam, between March and July 2024. Nasal swab samples were obtained through simple random sampling. Identification of *S. aureus* was performed using conventional microbiological techniques and confirmed via real-time PCR targeting the *nuc* gene. Initial carriers underwent repeat sampling at three and six weeks to classify carriage patterns: persistent carriers (positive in all three samples), intermittent carriers (positive in one or two samples), and non-carriers (negative at baseline). Risk factors were assessed through a structured questionnaire encompassing demographic data, medical history, and hygiene practices. Statistical analysis was conducted using the chi-square test and Fisher's exact test, with a significance threshold of $\alpha = 0.05$. The overall prevalence of *S. aureus* nasal carriage was 12.5% (18/144; 95% CI: 7.8–19.0%). Among these, 55.6% (10/18) were classified as persistent carriers, while 44.4% (8/18) were intermittent carriers. Pre-clinical students exhibited a marginally higher carriage rate than clinical students (13.5% vs 11.4%, $p = 0.705$). No statistically significant associations were observed between carriage status and demographic variables (age, gender, ethnicity), recent medical history (illness, antibiotic usage), or hygiene practices (all $p > 0.05$). The study's findings, however, are limited by the relatively small sample size, which may have hindered the detection of clinically meaningful associations. This study establishes a baseline *S. aureus* nasal carriage prevalence of 12.5% among Malaysian MLT students, with approximately equal distribution between persistent and intermittent carriage patterns. The absence of significant risk factor associations may reflect limited statistical power rather than true absence of relationships. These preliminary findings warrant larger, multi-centre studies with adequate power to identify meaningful risk factors and inform evidence-based infection prevention strategies in healthcare educational settings.

Keywords:

Staphylococcus aureus, nasal carriage, carriage patterns, risk factors, Medical Laboratory Technology students

Corresponding author:

mohdfahmi@uitm.edu.my

INTRODUCTION

Staphylococcus aureus is a versatile pathogen responsible for a broad spectrum of infections, ranging from superficial skin and soft tissue infections to life-threatening invasive diseases including pneumonia, endocarditis, and sepsis (Tong et al., 2015). As both a commensal organism and opportunistic pathogen, *S. aureus* asymptotically colonises the anterior nares of approximately 20–30% of healthy individuals, establishing a dynamic relationship between host and microorganism that can persist for months to years (Krismer et al., 2017).

The epidemiology of *S. aureus* nasal carriage demonstrates considerable heterogeneity across populations and geographic regions. Contemporary longitudinal studies consistently identify three distinct carriage patterns: persistent carriers (approximately 20–24% of individuals),

who maintain consistent nasal colonisation over extended periods; intermittent carriers (30–32%), who demonstrate variable colonisation patterns with periods of positivity and negativity; and non-carriers (approximately 50%), who rarely harbour the organism (Young et al., 2017; van Belkum et al., 2009). This classification system, based on longitudinal sampling over 12–24 weeks, has proven clinically relevant as persistent carriers demonstrate significantly higher risks for subsequent *S. aureus* infections compared to intermittent carriers or non-carriers (Nouwen et al., 2005).

Recent investigations have refined our understanding of carriage dynamics through advanced molecular typing methods. Studies employing multi-locus sequence typing (MLST) and *spa* typing have revealed that persistent carriers typically maintain genetically identical strains for

months, with the median duration of persistent carriage extending beyond 12 weeks, whilst intermittent carriers show more frequent strain turnover with a median carriage duration of 4 weeks between episodes (Lebon et al., 2015; Young et al., 2017). These molecular insights have important implications for infection prevention strategies, as persistent carriers represent a more stable reservoir for transmission.

Healthcare environments present unique challenges for *S. aureus* transmission and infection control. Healthcare workers (HCWs) and students in healthcare programmes face elevated exposure risks through direct patient contact and handling of clinical specimens, potentially serving as reservoirs for nosocomial transmission (Danelli et al., 2020). Recent studies have documented variable carriage rates amongst healthcare personnel, ranging from 25-43% depending on specialty, geographic location, and infection control practices (Desta et al., 2022; El Aila et al., 2017). Of particular concern is the persistence of methicillin-resistant *S. aureus* (MRSA) in healthcare settings, with studies reporting MRSA carriage rates of 8-15% amongst healthcare workers globally (Anbazhagan et al., 2020).

Medical laboratory technology (MLT) students represent a distinct population within healthcare education, characterised by intensive exposure to clinical specimens and pathogenic microorganisms during their training. Unlike other healthcare students who primarily engage in patient care activities, MLT students routinely handle *S. aureus* isolates during laboratory practicum sessions, potentially increasing their colonisation risk. Recent evidence suggests that students may have higher carriage rates than established healthcare workers, with one Brazilian study reporting *S. aureus* carriage rates of 42.9% amongst healthcare students and workers, with male students showing 1.8-fold higher odds of carriage (Danelli et al., 2020).

Regional epidemiological data from Southeast Asia reveal considerable variation in *S. aureus* carriage rates and resistance patterns. Studies from Malaysia have reported prevalence rates ranging from 9-28% amongst various healthcare populations, with MRSA prevalence varying between 2-14% across different institutions (Rampal et al., 2020; Anbazhagan et al., 2020). A 2020 systematic review examining Malaysian healthcare students found increasing carriage rates with advancing academic years and hospital exposure, highlighting the importance of understanding carriage dynamics in educational settings (Lemos et al., 2021). However, comprehensive data specifically addressing carriage patterns and risk factors amongst Malaysian healthcare students, particularly those in laboratory medicine programmes, remain limited.

Understanding the factors associated with *S. aureus* nasal carriage has important implications for infection prevention and control strategies. Recent research has identified several potential risk factors, including demographic characteristics (age, gender, ethnicity), medical history (recent antibiotic use, underlying medical conditions), hygiene practices, and healthcare exposure patterns (Mehraj et al., 2016). However, the relative importance of these factors varies considerably across populations and settings, necessitating population-specific studies to inform targeted interventions. Contemporary studies have also highlighted the role of host genetic factors and nasal microbiome composition in determining carriage susceptibility (Accorsi et al., 2020).

The present study was designed to address critical knowledge gaps regarding *S. aureus* epidemiology amongst Malaysian healthcare students. Specifically, we aimed to: (1) determine the prevalence of *S. aureus* nasal carriage amongst pre-clinical and clinical MLT students at a major Malaysian university; (2) characterise carriage patterns using longitudinal sampling to classify students as persistent, intermittent, or non-carriers; (3) identify demographic, medical, and behavioural risk factors associated with *S. aureus* colonisation; and (4) compare carriage rates between pre-clinical students (limited pathogen exposure) and clinical students (extensive laboratory exposure).

The findings from this investigation will contribute baseline epidemiological data for this understudied population and may inform the development of evidence-based infection prevention strategies within healthcare educational institutions in Malaysia and similar settings. Furthermore, understanding carriage patterns amongst future laboratory professionals may help optimise screening protocols and protective measures for this high-risk occupational group, particularly given the evolving landscape of antimicrobial resistance in the region.

MATERIALS AND METHODS

Sample size calculation

Sample size calculation was based on the formula for cross-sectional prevalence studies (Naing et al., 2006): $n = Z^2\alpha/2 \times P(1-P) / d^2$, where n is the required sample size, $Z_{\alpha/2}$ is the standard normal variate (1.96 for 95% confidence interval), P is the expected prevalence of *S. aureus* nasal carriage, and d is the absolute precision desired. Based on previous Malaysian studies reporting *S. aureus* carriage rates of 20-30% among healthcare populations (Rampal et al., 2020), we assumed a

prevalence of 25% with a precision of $\pm 7\%$ and 95% confidence level. This yielded a minimum sample size of 147 participants. Accounting for a 10% non-response rate, the target sample size was 163 participants. A total of 144 students were successfully recruited (96.7% of target), providing adequate statistical power for prevalence estimation.

Study design and population

The overall sampling methodology and participant flow is illustrated in Figure 1. A prospective cross-sectional study with longitudinal follow-up was conducted at UiTM Puncak Alam from March to July 2024. Using simple random sampling, 144 MLT students were recruited and categorised into pre-clinical (semesters 1-4) and clinical (semesters 5-8) groups based on their level of laboratory pathogen exposure. Sample size was calculated based on an estimated *S. aureus* prevalence of 25% with 7% precision and 95% confidence level, accounting for 10% non-response rate. All participants provided written informed consent, and those with recent antibiotic use (within 4 weeks) or acute respiratory infections were excluded.

A structured questionnaire adapted from previous validated studies was administered to collect demographic data (age, gender, ethnicity, academic semester), medical factors (recent illness, antibiotic use, hospitalisation history), and hygiene practices (hand hygiene frequency, nose-touching habits). The questionnaire was pilot-tested and culturally adapted for the Malaysian student population.

To determine nasal carriage patterns of *S. aureus*, participants initially identified as carriers during baseline sampling (week 0) underwent additional nasal swab collections at weeks 3 and 6. Based on these results, students were classified as non-carriers (negative at baseline), intermittent carriers (positive in 1-2 samples), or persistent carriers (positive in all three samples). This classification follows established criteria from longitudinal carriage studies and provides clinically relevant stratification.

Sample collection maybe to include flowchart on participant recruitment , exclusions and others

Nasal specimens were collected at the Pathogen Laboratory, Faculty of Health Sciences, UiTM Puncak Alam by trained research personnel using standardised aseptic technique. A sterile cotton-tipped swab moistened with

sterile phosphate-buffered saline (PBS) was inserted approximately 1-2 cm into the anterior nasal vestibule of the right nostril, gently rotated for 10 seconds, and immediately transported in Amies transport medium to the laboratory within two hours. All samples were processed within 24 hours of collection to ensure viability. Identification of *S. aureus*

S. aureus identification employed both conventional microbiological methods and molecular confirmation. Primary isolation was performed by streaking nasal swabs onto Columbia blood agar and mannitol salt agar, followed by incubation at 37°C for 24-48 hours. Presumptive identification was based on colony morphology (golden-yellow pigmentation, β -haemolysis), positive catalase test, positive tube coagulase test using rabbit plasma, and mannitol fermentation on mannitol salt agar.

Molecular confirmation utilised SYBR Green-based real-time PCR targeting the species-specific thermonuclease (*nuc*) gene. Bacterial DNA was extracted using the boiling method: overnight colonies were suspended in sterile distilled water, boiled at 100°C for 10 minutes, then centrifuged at 12,000 \times g for 5 minutes. The qPCR reaction (25 μ L total volume) contained SYBR Green Master Mix, specific primers (*nuc*-F: 5'-GCG ATT GAT GGT GAT ACG GTT-3' and *nuc*-R: 5'-AGC CAA GCC TTG ACG AAC TAA AGC-3'), and template DNA. Thermal cycling comprised initial denaturation at 95°C for 10 minutes, followed by 40 cycles of 95°C (15 seconds), 52.6°C (30 seconds), and 72°C (30 seconds), with melting curve analysis from 65°C to 95°C. *S. aureus* ATCC 25923 served as positive control for all identification procedures.

Quality control measures included appropriate positive and negative controls for all tests, blinded laboratory analysis, and adherence to standard microbiological protocols. Laboratory personnel were masked to participant clinical data to minimise analytical bias.

Statistical analyses

Data analysis was performed using SPSS version 28.0. *S. aureus* nasal carriage prevalence was calculated as the proportion of participants with positive baseline cultures, with 95% confidence intervals determined using the Wilson score method. Carriage patterns (persistent, intermittent, non-carrier) were analysed after completion of longitudinal sampling.

Associations between *S. aureus* carriage status, carriage patterns, and potential risk factors were assessed using Pearson's chi-square test for categorical variables with expected cell counts ≥ 5 , and Fisher's exact test when expected counts were < 5 . Independent samples t-test or

Mann-Whitney U test were used for continuous variables, depending on data distribution. Statistical significance was set at $p \leq 0.05$, with all tests being two-tailed.

Post-hoc power analysis was conducted to assess the study's ability to detect meaningful associations given the observed prevalence and sample size. Effect sizes were calculated using Cramér's V for categorical associations, and results were interpreted within the context of clinical significance and study limitations. Missing data patterns were evaluated, and complete case analysis was performed with sensitivity analyses to assess the impact on study conclusions.

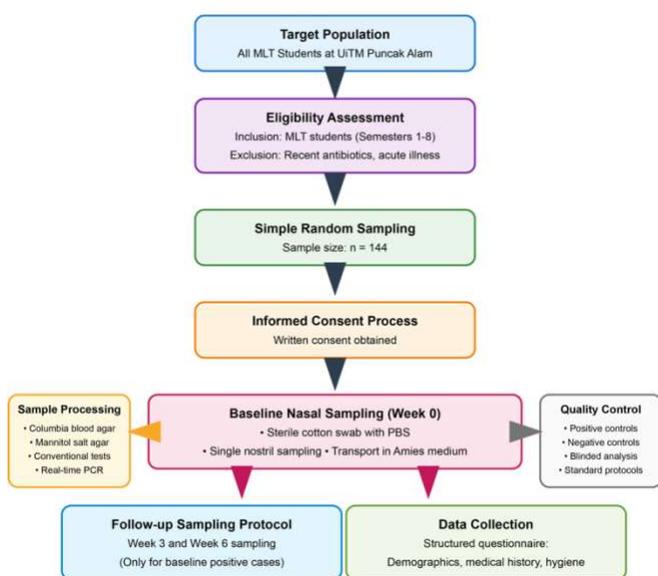


Figure 1. Sampling methodology flowchart for *S. aureus* nasal carriage study among MLT students.

Ethics approval

This study was reviewed and approved by the UiTM Research Ethics Committee (Reference: REC/170/24), ensuring compliance with the Declaration of Helsinki and Malaysian research guidelines. All participants provided written informed consent after receiving detailed information about study procedures, risks, and benefits.

RESULTS

Study Population Characteristics

A total of 144 MLT students participated in the study, comprising 74 pre-clinical students (51.4%) and 70 clinical students (48.6%). The overall response rate was 96.7% (144/149 eligible students). Participant demographics showed a predominance of female students (123/144, 85.4%) and Malay ethnicity (138/144, 95.8%). The mean

age was 22.1 ± 1.8 years (range: 19-28 years), with no significant age difference between pre-clinical and clinical groups (22.0 ± 1.7 vs 22.2 ± 1.9 years, $p = 0.521$).

S. aureus Nasal Carriage Prevalence

The study identified a nasal carriage prevalence of 12.5% for *Staphylococcus aureus* among Malaysian MLT students, based on microbiological analysis using conventional identification methods and molecular confirmation via real-time PCR targeting the *nuc* gene. Out of 144 participants, 18 tested positive (12.5%, 95% CI: 7.8-19.0%), with 100% concordance between conventional methods and PCR, as all phenotypically identified isolates were confirmed as *S. aureus*. Molecular confirmation was achieved through real-time PCR targeting the *nuc* gene, demonstrating 100% concordance with conventional identification methods (Figure 2)

While pre-clinical students showed a slightly higher carriage rate (13.5%) compared to clinical students (11.4%), this difference was not statistically significant ($\chi^2 = 0.145$, $df = 1$, $p = 0.705$). Similarly, no significant differences in carriage rates were found between genders (male: 9.5% vs female: 13.0%, $p = 1.000$) or ethnic groups (Malays: 12.3% vs others: 16.7%, $p = 0.558$).

The study found that persistent carriage was slightly more common than intermittent carriage (55.6% vs 44.4%) among carriers. However, no significant associations were identified between carriage status and demographic, medical, or hygiene-related risk factors. The study's statistical power for detecting modest associations was limited by the relatively small number of carriers identified.

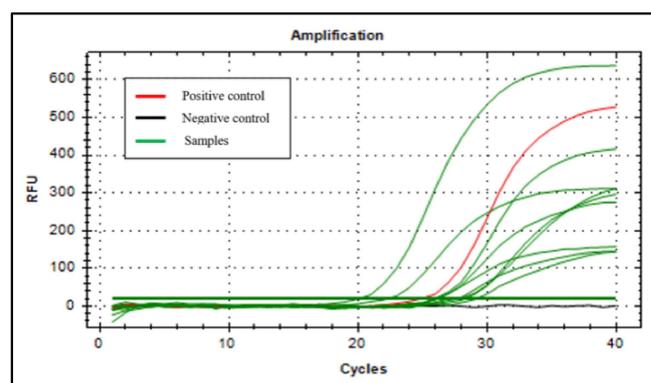


Figure 2: Molecular confirmation of *S. aureus* isolates by real-time PCR targeting the *nuc* gene. Amplification curves showing positive samples (green lines) with exponential fluorescence increase and negative samples (black lines) with no amplification. Positive control (*S. aureus* ATCC 25923) and negative control included. All 18 phenotypically identified isolates were confirmed as *S.*

aureus by PCR (100% concordance).

Carriage Pattern Classification

Among the 18 baseline carriers who underwent longitudinal follow-up sampling at weeks 3 and 6, carriage patterns were successfully determined for all participants with 100% compliance to follow-up visits. Based on the longitudinal sampling results, 10 students (55.6%) were classified as persistent carriers (positive in all three samples), whilst 8 students (44.4%) were classified as intermittent carriers (positive in 1-2 samples over the three time points). No participants initially classified as non-carriers (negative at baseline) developed carriage during the follow-up period. The distribution of carriage patterns among the 18 baseline carriers is presented in Figure 3.

The distribution of persistent carriers was similar between pre-clinical and clinical groups (6/10, 60.0% vs 4/8, 50.0% of carriers, respectively, $p = 0.692$, Fisher's exact test). Demographic characteristics of carrier types showed that persistent carriers comprised 1 male (10.0%) and 9 females (90.0%) with a mean age of 22.1 ± 1.6 years, whilst intermittent carriers included 1 male (12.5%) and 7 females (87.5%) with a mean age of 22.3 ± 2.1 years. No significant associations were observed between carriage patterns and demographic variables (all $p > 0.05$).

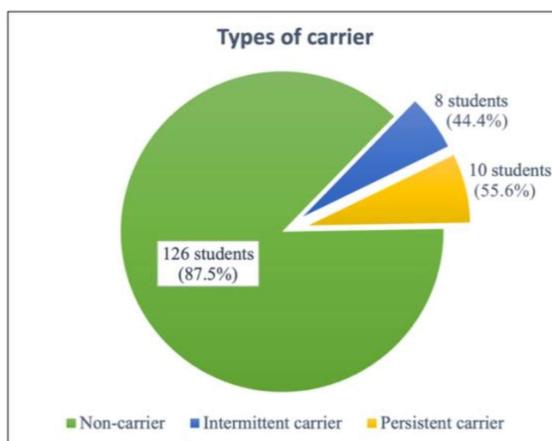


Figure 3: The distribution and percentages of *S. aureus* carrier types among MLT students in UiTM Puncak Alam.

Risk Factor Analysis

Univariate analysis of potential risk factors for *S. aureus* nasal carriage revealed no statistically significant associations with any of the examined variables (Table 1). Among carriers, 2/18 (11.1%) reported a history of cold and fever in the preceding two weeks compared to 37/126

(29.4%) among non-carriers, though this difference was not statistically significant ($p = 0.155$, Fisher's exact test). Notably, none of the carriers reported recent antibiotic use or presence of unhealed wounds, whilst 4/126 (3.2%) non-carriers reported antibiotic use and 3/126 (2.4%) reported unhealed wounds in the preceding two weeks.

Regarding hygiene practices, 9/18 (50.0%) carriers reported frequently touching their nose compared to 50/126 (39.7%) non-carriers ($p = 0.405$, χ^2 test). Interestingly, a higher proportion of carriers reported frequently washing hands after touching their nose (5/18, 27.8%) compared to non-carriers (18/126, 14.3%), though this difference did not reach statistical significance ($p = 0.168$, Fisher's exact test). The association between potential risk factors and *S. aureus* nasal carriage is summarised in Table 1.

When stratified by carriage patterns, no significant differences were observed between persistent and intermittent carriers for any risk factors examined (all $p > 0.05$). The small sample sizes within each carriage pattern group limited the statistical power to detect meaningful associations.

Table 1: Association between potential risk factors and *S. aureus* nasal carriage among MLT students

Variable	<i>S. aureus</i> carrier (n=18)	<i>S. aureus</i> non-carrier (n=126)	P-value
Demographics			
Male gender	2 (11.1%)	19 (15.1%)	1.000 ^b
Malay ethnicity	17 (94.4%)	121 (96.0%)	0.558 ^b
Clinical status	8 (44.4%)	62 (49.2%)	0.705 ^a
Medical history			
Cold/fever (past 2 weeks)	2 (11.1%)	37 (29.4%)	0.155 ^b
Antibiotic use (past 2 weeks)	0 (0.0%)	4 (3.2%)	1.000 ^b
Unhealed wounds	0 (0.0%)	3 (2.4%)	1.000 ^b
Hygiene practices			
Frequently touch nose	9 (50.0%)	50 (39.7%)	0.405 ^a
Hand washing after nose touching	5 (27.8%)	18 (14.3%)	0.168 ^b

DISCUSSION

This study presents significant baseline data on the nasal carriage patterns of *Staphylococcus aureus* among medical

laboratory technology (MLT) students in Malaysia, highlighting a population that has unique occupational exposure risks and has been under-researched in the regional literature. Our findings indicate a carriage prevalence of 12.5%, with a near-equal distribution between persistent and intermittent carriers, and no significant associations identified with the examined risk factors.

Prevalence in Regional and Global Context

The observed nasal carriage prevalence of *S. aureus* (12.5%) among MLT students at UiTM Puncak Alam falls within the lower range of prevalence rates reported in similar populations globally. This finding is consistent with other Malaysian studies involving occupational exposure populations, including a 12% carriage rate among dairy farm workers (Saeed et al., 2022) and 13% among animal handlers (Chai et al., 2022). However, our prevalence rate is considerably lower than that documented among healthcare students in other Malaysian institutions, including 26% among medical and health sciences students at Universiti Putra Malaysia (Jaganath et al., 2018) and 23.4% among students and staff at the same institution (Alahmadi et al., 2023).

Internationally, neighboring regions report higher prevalences; for example, a study from Thailand noted a carriage rate of 25.7% among healthcare workers (Jaganath et al., 2018), while Brazil reported a striking 42.9% among healthcare students and workers (Yilmaz et al., 2016). Conversely, certain temperate regions observe similar or lower rates, such as 10% prevalence among medical students in specific European settings (Garedew et al., 2015). These discrepancies in carriage rates can be attributed to various factors, including environmental conditions, demographic genetics, hygiene practices, exposure to healthcare settings, and methodological differences in the studies conducted (Kahanov et al., 2015).

The relatively low prevalence identified in our study may be linked to several distinctive elements of the Malaysian healthcare education system. These include comprehensive infection control training programs, mandatory use of personal protective equipment during laboratory training, and cultural norms promoting regular hand hygiene. Additionally, the timing of the study during the COVID-19 pandemic may have bolstered hygiene practices, limiting transmission, though this aspect was beyond the scope of our evaluation (Kumbhar et al., 2018).

Carriage Pattern Distribution and Clinical Significance

Our longitudinal investigation unveiled that among the 18 identified carriers, 55.6% exhibited persistent carriage patterns while 44.4% displayed intermittent carriage. This distribution contrasts with classical epidemiological data, which typically report approximately 20% persistent carriers and 30% intermittent carriers within the general population (Alrabiah et al., 2016). The heightened ratio of persistent carriers in our findings may reflect the specific demographics of young adults or may be influenced by the unique exposure environment faced by students of laboratory medicine (Du et al., 2022).

The clinical implications of these patterns are profound, as persistent carriers are known to exhibit a 2–9-fold increased risk of developing *S. aureus* infections compared to their intermittent counterparts or non-carriers (Yulianto et al., 2023). Moreover, persistent carriers often serve as stable reservoirs for transmission, thereby augmenting the potential for environmental dispersal of *S. aureus* (Jaganath et al., 2018). Given that laboratory medicine students routinely handle clinical specimens potentially containing this pathogen, understanding carriage dynamics is pivotal for devising targeted decolonization strategies and infection prevention protocols.

Comparison Between Pre-clinical and Clinical Students

In contrast to our original hypothesis, pre-clinical students presented a marginally higher carriage rate (13.5%) than clinical students (11.4%), albeit with no statistical significance ($p = 0.705$). This observation challenges the findings of prior studies that indicate a positive correlation between increased healthcare exposure and carriage rates (Hirose et al., 2021; Chai et al., 2022; . However, our data are compatible with research suggesting that the relationship between healthcare exposure and *S. aureus* carriage may be multifaceted and not necessarily linear (Alhunaif et al., 2021; Regasa et al., 2019).

Several hypotheses could elucidate this unexpected result. For instance, clinical students might have developed superior infection control protocols through their extensive training and practical experience. Alternatively, there might be a phenomenon of "acquired immunity" from repeated exposure that reduces susceptibility to colonization among more experienced students (Deyno et al., 2017). Furthermore, a heightened awareness concerning personal protective equipment usage and hand hygiene among clinical students may contribute to these findings. Ultimately, these results underscore the possibility that inherent host characteristics (e.g., genetic

dispositional factors, immune responses, nasal microbiome variations) may significantly outweigh merely exposure-related determinants in influencing carriage rates (Alahmadi et al., 2023).

Risk Factor Analysis and Methodological Considerations

Our meticulous risk factor analysis revealed no significant correlations between *S. aureus* carriage and demographic, medical, or hygiene variables. The interpretation of these negative findings must be approached with caution, especially considering the statistical power limitations of our study. A post-hoc power analysis indicated that our study could detect odds ratios of 3.5 or greater with 80% power, implying that smaller yet potentially clinically pertinent associations may have been overlooked (Aurangzeb et al., 2023).

Notably, various trends of interest emerged, although they did not achieve statistical significance. Non-carriers reported a higher frequency of recent symptoms such as cold and fever (29.4% vs 11.1%), insinuating that recent upper respiratory infections might paradoxically correlate with decreased nasal carriage. This could possibly point to competitive exclusion by other pathogens or enhanced local immune responses following infections, though this interpretation remains speculative (Briscoe et al., 2019). Moreover, carriers indicated a higher tendency to wash hands following contact with their nose (27.8% vs 14.3%), which could suggest that individuals predisposed to nasal carriage might be more attuned to their behaviors regarding nose touching and hygiene practices, despite those measures inadequately preventing colonization (Mergenhausen et al., 2020).

Interestingly, the lack of significant demographic associations resonates with certain other studies but diverges from those that report gender or age-related differences. The predominance of female participants in our study (85.4%) may impede the detection of gender correlations, and the constrained age range (mostly 19–28 years) may limit the identification of age-related variations that manifest across broader demographics (Saeed et al., 2022).

Study Strengths and Limitations

This study possesses multiple methodological strengths that bolster the validity of the findings. The incorporation of traditional microbiological techniques alongside molecular confirmation via real-time PCR ensured accurate species identification with 100% concordance. Employing a longitudinal approach fortified carriage

classification, and the implementation of standardized sampling protocols effectively minimized technical variability. Furthermore, blinded laboratory analyses mitigated potential biases in result interpretation (Zeryehun & Abera, 2017).

Nevertheless, certain limitations must be acknowledged. The single-center design constrains the generalizability of our findings to different institutions or regions with disparate student populations or curricula. Additionally, our sample size, while adequate for estimating prevalence, was insufficient to detect modest associations with risk factors. The reliance on single nostril sampling may have also led to an underestimation of true carriage prevalence, as bilateral sampling typically enhances detection sensitivity (Commons et al., 2015). Moreover, the six-week timeframe for assessing carriage patterns may be seen as relatively limited for definitive classification, with recommendations advocating for observation periods of 12–24 weeks in some studies (DePalma et al., 2022). Finally, the cross-sectional elements of our risk factor assessments curtail causal inference, and potential recall bias may influence self-reported behavior and medical histories.

Implications for Infection Prevention and Control

The findings of this study carry several practical implications for infection prevention strategies within healthcare educational environments. Identifying persistent carriers within the student population suggests that targeted screening and decolonization initiatives could be advantageous, particularly for students poised to enter higher-risk clinical scenarios. However, a careful evaluation of the cost-effectiveness of such programs is warranted, given the overall low prevalence observed.

Furthermore, the absence of significant differences between pre-clinical and clinical students indicates a need for early implementation of infection control education throughout healthcare programs, instead of concentrating solely during clinical stages. The fact that traditional risk factors did not effectively predict carriage in our demographics suggests that uniform precautions and comprehensive infection control strategies need to be emphasized for all students, irrespective of perceived risk profiles.

Future Research Directions

Our research highlights numerous critical avenues for future investigation. Larger, multi-center studies with adequate statistical power are required to definitively

elucidate risk factors associated with *S. aureus* carriage among healthcare students in Malaysia. Longitudinal studies characterized by extended follow-up periods could yield more robust definitions of carriage patterns and assess the durability of carriage status over time.

Moreover, future inquiries should delve into the molecular epidemiology of *S. aureus* strains within healthcare student cohorts, accompanied by investigations into antimicrobial resistance patterns and virulence factor profiles. Understanding the nasal microbiome and the genetic determinants of host susceptibility could further illuminate the complexity of carriage dynamics. Additionally, intervention studies that evaluate the effectiveness of targeted decolonization strategies within student populations would furnish valuable evidence for guiding infection prevention policies.

CONCLUSION

This study establishes a baseline prevalence of *S. aureus* nasal carriage at 12.5% among Malaysian MLT students, revealing a more significant proportion of persistent carriers than typically observed in the general population. The absence of notable associations with conventional risk factors underscores the intricate nature of carriage determinants, suggesting that host-related factors may overshadow exposure-related variables in this cohort. These findings provide essential baseline data for informing evidence-based infection prevention strategies in healthcare educational settings, emphasizing the need for larger, multi-center studies to enhance our understanding of carriage dynamics within this pivotal population.

ACKNOWLEDGEMENT

The authors wish to express their gratitude to the Centre for Medical Laboratory Studies for their unwavering support throughout the course of this study. Additionally, they extend their sincere appreciation to the laboratory staff for their invaluable cooperation and assistance during the study.

REFERENCES

- Alahmadi, T., Alahmadey, Z., Elbanna, K., Neyaz, L., Ahmad, I., & Abulreesh, H. (2023). The prevalence and clinical characteristics of multidrug-resistant hospital-acquired staphylococcus aureus in medina, saudi arabia. *Journal of Pure and Applied Microbiology*, 17(1), 499-514. <https://doi.org/10.22207/jpam.17.1.44>
- Alhunaif, S., Almansour, S., Almutairi, R., Alshammari, S., Alkhonain, L., Alalwan, B., ... & Alothman, A. (2021). Methicillin-resistant staphylococcus aureus bacteremia: epidemiology, clinical characteristics, risk factors, and outcomes in a tertiary care center in riyadh, saudi arabia. *Cureus*. <https://doi.org/10.7759/cureus.14934>
- Arabiah, K., Alola, S., Banyan, E., Shaalan, M., & Johani, S. (2016). Characteristics and risk factors of hospital acquired — methicillin-resistant staphylococcus aureus (ha-mrsa) infection of pediatric patients in a tertiary care hospital in riyadh, saudi arabia. *International Journal of Pediatrics and Adolescent Medicine*, 3(2), 71-77. <https://doi.org/10.1016/j.ijpam.2016.03.006>
- Anbazhagan, D., Hui, M. J., Aisyah, N., Syazwani, A., Keong, T. P., Shuen, L. J., Sundram, M. S., Subramaniam, H., & Kumarasamy, V. (2020). Nasal carriage of methicillin-resistant Staphylococcus aureus among healthcare undergraduates in Malaysia. *International Research Journal of Medicine and Medical Sciences*, 8(4), 116-118. DOI: 10.30918/IRJMMS.84.20.042
- Aurangzeb, A., Khan, M., Khokhar, R., Pasha, W., Sathio, S., & Ramchand, R. (2023). Bacterial spectrum and antibiotic susceptibility among hospitalized pediatric cardiac patients. *International Journal of Health Sciences*, 7(S1), 2397-2403. <https://doi.org/10.53730/ijhs.v7ns1.14517>
- Briscoe, C., Reich, P., Fritz, S., & Coughlin, C. (2019). Staphylococcus aureus antibiotic susceptibility patterns in pediatric atopic dermatitis. *Pediatric Dermatology*, 36(4), 482-485. <https://doi.org/10.1111/pde.13867>
- Chai, M., Sukiman, M., Baharin, A., Ramlan, I., Lai, L., Liew, Y., ... & Ghazali, M. (2022). Methicillin-resistant staphylococcus aureus from peninsular malaysian animal handlers: molecular profile, antimicrobial resistance, immune evasion cluster and genotypic categorization. *Antibiotics*, 11(1), 103. <https://doi.org/10.3390/antibiotics11010103>
- Commons, R., Robinson, C., Gawler, D., Davis, J., & Price, R. (2015). High burden of diabetic foot infections in the top end of australia: an emerging health crisis (define study). *Diabetes Research and Clinical Practice*, 110(2), 147-157. <https://doi.org/10.1016/j.diabres.2015.09.016>
- Danelli, T., Duarte, F. C., de Oliveira, T. A., da Silva, R. S., Frizon Alfieri, D., Gonçalves, G. B., de Oliveira, C. F.,

- Tavares, E. R., Yamauchi, L. M., Perugini, M. R. E., & Yamada-Ogatta, S. F. (2020). Nasal Carriage by *Staphylococcus aureus* among Healthcare Workers and Students Attending a University Hospital in Southern Brazil: Prevalence, Phenotypic, and Molecular Characteristics. *Interdisciplinary perspectives on infectious diseases*, 2020, 3808036. <https://doi.org/10.1155/2020/3808036>
- DePalma, B., Nandi, S., Chaudhry, W., Lee, M., Johnson, A., & Doub, J. (2022). Assessment of staphylococcal clinical isolates from periprosthetic joint infections for potential bacteriophage therapy. *The Journal of Bone and Joint Surgery (American)*, 104(8), 693-699. <https://doi.org/10.2106/jbjs.21.00958>
- Desta, K., Aklillu, E., Gebrehiwot, Y., Enquesselassie, F., Cantillon, D., Al-Hassan, L., Price, J. R., Newport, M. J., Davey, G., & Woldeamanuel, Y. (2022). High Levels of Methicillin-Resistant *Staphylococcus aureus* Carriage Among Healthcare Workers at a Teaching Hospital in Addis Ababa Ethiopia: First Evidence Using mecA Detection. *Infection and drug resistance*, 15, 3135–3147. <https://doi.org/10.2147/IDR.S360123>
- Deyno, S., Fekadu, S., & Astatkie, A. (2017). Resistance of *staphylococcus aureus* to antimicrobial agents in ethiopia: a meta-analysis. *Antimicrobial Resistance and Infection Control*, 6(1). <https://doi.org/10.1186/s13756-017-0243-7>
- Du, F., Ma, J., Gong, H., Bista, R., Zha, P., Ren, Y., ... & Wang, C. (2022). Microbial infection and antibiotic susceptibility of diabetic foot ulcer in china: literature review. *Frontiers in Endocrinology*, 13. <https://doi.org/10.3389/fendo.2022.881659>
- El Aila, N. A., Al Laham, N. A., & Ayesh, B. M. (2017). Nasal carriage of methicillin resistant *Staphylococcus aureus* among health care workers at Al Shifa hospital in Gaza Strip. *BMC infectious diseases*, 17(1), 28. <https://doi.org/10.1186/s12879-016-2139-1>
- Garedew, L., Melese, B., & Tesfaye, R. (2015). *Staphylococcus aureus* in mastitic crossbreed cows and its associated risk factors in addis ababa city, ethiopia. *Ethiopian Veterinary Journal*, 19(1), 107. <https://doi.org/10.4314/evj.v19i1.1>
- Hirose, M., Aung, M., Fukuda, A., Yahata, S., Fujita, Y., Saitoh, M., ... & Kobayashi, N. (2021). Antimicrobial resistance and molecular epidemiological characteristics of methicillin-resistant and susceptible staphylococcal isolates from oral cavity of dental patients and staff in northern japan. *Antibiotics*, 10(11), 1316. <https://doi.org/10.3390/antibiotics10111316>
- Jaganath, D., Jorakate, P., Makprasert, S., Sangwichian, O., Akarachotpong, T., Thamthitawat, S., ... & Rhodes, J. (2018). *Staphylococcus aureus* bacteremia incidence and methicillin resistance in rural thailand, 2006–2014. *American Journal of Tropical Medicine and Hygiene*, 99(1), 155-163. <https://doi.org/10.4269/ajtmh.17-0631>
- Kahanov, L., Kim, Y., Eberman, L., Dannelly, K., Kaur, H., & Ramalinga, A. (2015). *Staphylococcus aureus* and community-associated methicillin-resistant *staphylococcus aureus* (ca-mrsa) in and around therapeutic whirlpools in college athletic training rooms. *Journal of Athletic Training*, 50(4), 432-437. <https://doi.org/10.4085/1062-6050-49.3.96>
- Krismer, B., Weidenmaier, C., Zipperer, A., & Peschel, A. (2017). The commensal lifestyle of *Staphylococcus aureus* and its interactions with the nasal microbiota. *Nature Reviews Microbiology*, 15(11), 675-687. <https://doi.org/10.1038/nrmicro.2017.104>
- Kumbhar, M., Kandhro, S., Rind, R., Kaleri, R., Chandio, I., Farooq, T., ... & Kumar, M. (2018). Prevalence and incidence of *staphylococcus aureus* from wound of different animal species. *Journal of Basic & Applied Sciences*, 14, 12-16. <https://doi.org/10.6000/1927-5129.2018.14.03>
- Lebon, A., Labout, J. A., Verbrugh, H. A., Jaddoe, V. W., Hofman, A., van Wamel, W., Moll, H. A., & van Belkum, A. (2008). Dynamics and determinants of *Staphylococcus aureus* carriage in infancy: the Generation R Study. *Journal of Clinical Microbiology*, 46(10), 3517-3521. <https://doi.org/10.1128/JCM.00641-08>
- Lemos, A. d. S., Souza, A. C. M. F. d., Karas, B., Calixto, C. M., Meijerink, C. I., Nascimento, F. C., ... & Montes, E. G. (2021). Prevalence of *staphylococcus aureus* and mrsa among medical students: a literature review. *Research, Society and Development*, 10(11), e347101119536. <https://doi.org/10.33448/rsd-v10i11.19536>
- Mehraj, J., Witte, W., Akmatov, M. K., Layer, F., Werner, G., & Krause, G. (2016). Epidemiology of *Staphylococcus aureus* nasal carriage patterns in the community. *Current Topics in Microbiology and*

- Immunology, 398, 55-87. https://doi.org/10.1007/82_2016_497
- Mergenhagen, K., Croix, M., Starr, K., Sellick, J., & Lesse, A. (2020). Utility of methicillin-resistant staphylococcus aureus nares screening for patients with a diabetic foot infection. *Antimicrobial Agents and Chemotherapy*, 64(4). <https://doi.org/10.1128/aac.02213-19>
- Naing, L., Winn, T., & Rusli, B. N. (2006). Practical issues in calculating the sample size for prevalence studies. *Archives of Orofacial Sciences*, 1, 9-14.
- Nouwen, J. L., Fieren, M. W., Snijders, S., Verbrugh, H. A., & van Belkum, A. (2005). Persistent (not intermittent) nasal carriage of *Staphylococcus aureus* is the determinant of CPD-related infections. *Kidney International*, 67(3), 1084-1092. <https://doi.org/10.1111/j.1523-1755.2005.00174.x>
- Rampal, S., Zainuddin, N. H., Elias, N. A., Tengku Jamaluddin, T. Z. M., Maniam, S., Teh, S. W., & Subbiah, S. K. (2020). Colonization of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among Medical Students in Tertiary Institution in Central Malaysia. *Antibiotics (Basel, Switzerland)*, 9(7), 382. <https://doi.org/10.3390/antibiotics9070382>
- Regasa, S., Mengistu, S., & Abraha, A. (2019). Milk safety assessment, isolation, and antimicrobial susceptibility profile of *staphylococcus aureus* in selected dairy farms of mukaturi and sululta town, oromia region, ethiopia. *Veterinary Medicine International*, 2019, 1-11. <https://doi.org/10.1155/2019/3063185>
- Saeed, S., Yazid, K., Hashimy, H., Dzulkifli, S., Nordin, F., Him, N., ... & Kamaruzzaman, N. (2022). Prevalence, antimicrobial resistance, and characterization of *staphylococcus aureus* isolated from subclinical bovine mastitis in east coast malaysia. *Animals*, 12(13), 1680. <https://doi.org/10.3390/ani12131680>
- Tong, S. Y. C., Davis, J. S., Eichenberger, E., Holland, T. L., & Fowler, V. G., Jr. (2015). *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clinical Microbiology Reviews*, 28(3), 603-661. <https://doi.org/10.1128/CMR.00134-14>
- van Belkum, A., Verkaik, N. J., de Vogel, C. P., Boelens, H. A., Verveer, J., Nouwen, J. L., Verbrugh, H. A., & Wertheim, H. F. (2009). Reclassification of *Staphylococcus aureus* nasal carriage types. *Journal of Infectious Diseases*, 199(12), 1820-1826. <https://doi.org/10.1086/599119>
- Yılmaz, M., Elaldi, N., Balkan, İ., Arslan, F., Batirel, A., Bakıcı, M., ... & Mert, A. (2016). Mortality predictors of *staphylococcus aureus* bacteremia: a prospective multicenter study. *Annals of Clinical Microbiology and Antimicrobials*, 15(1). <https://doi.org/10.1186/s12941-016-0122-8>
- Young, B. C., Golubchik, T., Batty, E. M., Fung, R., Larners-Svensson, H., Votintseva, A. A., Miller, R. R., Godwin, H., Knox, K., Everitt, R. G., Iqbal, Z., Rimmer, A. J., Cule, M., Ip, C. L., Didelot, X., Harding, R. M., Donnelly, P., Peto, T. E., Crook, D. W., Bowden, R., & Walker, A. S. (2017). Evolutionary dynamics of *Staphylococcus aureus* during progression from carriage to disease. *Proceedings of the National Academy of Sciences*, 114(18), 4645-4650. <https://doi.org/10.1073/pnas.1617027114>
- Yulianto, N., Armiyanti, Y., Agustina, D., Hermansyah, B., & Utami, W. (2023). Analysis of milking hygiene and its association to *staphylococcus aureus* contamination in fresh cow milk. *Jurnal Kesehatan Lingkungan*, 15(4), 275-282. <https://doi.org/10.20473/jkl.v15i4.2023.275-282>
- Zeryehun, T., & Abera, G. (2017). Prevalence and bacterial isolates of mastitis in dairy farms in selected districts of eastern harrarghe zone, eastern ethiopia. *Journal of Veterinary Medicine*, 2017, 1-7. <https://doi.org/10.1155/2017/6498618>

Isolation and Identification of Bacteria in Air Conditioning Systems of Medical Laboratory Teaching Laboratories in UiTM Selangor

Wan Nazatul Shima Wan Imron Faizal, Emida Mohamed*, Azlin Sham Ramlee@Rambely, Norhisham Haron, Siti Nazrina Camalxaman

Centre for Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Background: Air conditioning systems provide comforts in various settings including buildings, offices, rooms, laboratories and hospitals. However, prolonged use of air conditioning can lead to the buildup of dust and biofilm in filters, vents, and grilles, which can harbour airborne pathogens. This can contribute to diseases such as "sick house syndrome" through transmission of bacteria from the air conditioner. Currently, the study on prevalence of pathogenic bacteria in the air conditioning system at laboratories in Malaysia is still limited. Therefore, this study aims to determine the presence of bacteria on air conditioning vents in five teaching laboratories; Cytology, Haematology, Microbiology, Molecular and Parasitology laboratories in the Faculty of Health Sciences, UiTM Selangor, Malaysia. **Methods:** Four dust samples from the vent of air conditioners in each laboratory were collected and then inoculated on Nutrient Agar (NA) and Blood Agar (BA). Basic microbiological tests including gram stain, spore stain, biochemical tests, and novobiocin sensitivity test were performed to identify the bacterial isolates. **Results:** A total of 23 bacterial isolates were obtained, and five genera were successfully identified; *Bacillus* spp., *Staphylococcus epidermidis*, *Clostridium* spp., *Paenibacillus* spp., and *Listeria* spp. These bacteria are known to potentially cause respiratory tract infections. **Conclusion:** Based on the findings, it can be concluded that the air conditioning vents in teaching laboratories are contaminated with various bacterial species that could pose a risk to health.

Keywords:

air conditioning systems; laboratory; bacteria; pathogenic bacteria

INTRODUCTION

Air conditioning system is a centralized equipment that creates a constant environment with controlled temperature, humidity, and purity (Anas et al., 2016). It has frequently been employed as cooling systems in buildings and is utilised in a variety of settings, including automobiles, offices, homes, and industries. The cooling sensation from air conditioner provides comfort towards human's life especially during hot weather. However, air conditioner has its own disadvantages such as costly compared to other appliances and consuming a lot of electricity. Most importantly, the public are unaware about the potential health risks associated with prolonged use of air conditioner (Govindasamy et al., 2014).

Risk of airborne pathogen transmission can be developed by the accumulation of dust and biofilm in air conditioning especially during air conditioner operation, contributing to indoor air contamination. Some of them may be systems such as filters, vents, and grilles. Microorganisms residing on these

surfaces can be released into the air, pathogenic if inhaled. According to a study by Anas et al. (2016), majority of air conditioners are using improper filters, which caused them to operate outside of their intended parameters. Additionally, they indicated that humidity and dust accumulation, particularly in the cooling equipment's downstream area, allowed bacteria such as *Bacillus* spp., *Staphylococcus* spp., and *Streptococcus* spp. to survive. Therefore, when users spend an excessive amount of time in an air-conditioned indoor environment, it has been observed that they may develop "sick house syndrome". Signs of "sick house syndrome" are headache, watery eyes, skin disorders, and weakness (Anas et al., 2016; Ayilara & Oginni, 2016). This notion is further supported by a study conducted in 2005 by Al Shahwani which demonstrated a correlation between "sick house syndrome" and contaminated air conditioning systems.

Malaysians should be particularly concerned, as the country's warm tropical climate encourages frequent use of air conditioners. This will increase the risk of exposure to biogenic indoor air pollutants, including allergens, mycotoxins, volatile organic compounds, bacteria, and molds (Chan et al., 2011). According to Chan et al. (2011), contaminated indoor air handling systems have been related to several outbreaks of air-borne illnesses such as aspergillosis, Legionnaires' disease, and severe acute respiratory syndrome. In 2011, Alangaden stated that organisms like *Staphylococcus* spp., *Streptococcus* spp.,

* Corresponding author.

E-mail address: emida894@uitm.edu.my

Micrococcus spp., *Bacillus spp.*, and *Serratia spp.*, were the most isolated airborne bacteria found in laboratory rooms, libraries, hospitals, and schools. They stated that high concentration of these microorganisms can lead to asthma, allergenic disease, respiratory and pulmonary infections. Another study conducted at academic institutions in South Western Nigeria discovered four organisms isolated from indoor air conditioner and grills which were *Bacillus spp.*, *Pseudomonas spp.*, *Streptococcus spp.*, and *Staphylococcus spp.* (Ayilara & Oginni, 2016). Recently, a study performed by Wopereis et al (2020) stated that controlling indoor air quality is crucial for reducing infections, especially in hospital settings where immunocompromised patients are more vulnerable to infection. Therefore, as preventive measures, this study was conducted to evaluate the presence of pathogenic bacteria on air conditioner vents in teaching laboratories at Centre for Medical Laboratory Technology Studies, Faculty of Health Science, Universiti Teknologi MARA, Puncak Alam Campus, Selangor.

MATERIALS AND METHODS

Sample Collection

The study was conducted during the months of March 2024 to July 2024. Collection of samples were conducted by taking dust samples from air conditioner vents in five Centre for Medical Laboratory Technology Studies teaching laboratories (Cytology, Haematology, Microbiology, Molecular & Parasitology). Four dust samples were collected from a single air-conditioning vent in each laboratory using sterile cotton swabs and cultured on Nutrient Agar (NA) as primary agar. All collections were performed under similar conditions during working hours with ventilation units in operation.

Isolation and Identification

The sterile cotton swabs used to collect dust samples from the air conditioner vents were soaked in tryptic soy broth. The samples were then incubated 24 hours at 37°C. After 24 hours of incubation, a 10-fold serial dilution was carried out prior to utilising the spread plate method to distribute the samples over the plate. Following the serial dilution procedure, 100 µl of the sample was pipetted into the center of the solidified Nutrient Agar (NA) and dispersed uniformly across its surface using an L-shaped bent glass rod. The plates were then incubated at 37°C for 24 hours or overnight (Wise, 2006). After the incubation, the viable bacteria in the sample grew into distinct visible colonies on the surface of the medium.

RESULTS

A total of twenty-three colonies were isolated using the spread method from the five laboratories (Cytology, Haematology, Microbiology, Molecular, and Parasitology). Colony morphology of the bacteria isolated on Nutrient and Blood agars were then recorded according to their respective laboratories and each individual isolate was tagged with a label to facilitate the identification process.

From the Cytology Laboratory, three distinct bacterial colonies were obtained, while the Haematology Laboratory yielded eight. The Microbiology Laboratory produced six different colonies, whereas from the Molecular Laboratory we managed to isolate one type of colony. Additionally, five distinct colonies were obtained from the Parasitology Laboratory. Further observations revealed that the twenty-three colonies obtained comprised of five distinct types. Characteristics of the five colonies are as shown in Table 1 and Figure 1. Presence of the isolated bacteria in the air conditioner vents of the five laboratories are demonstrated in Table 2.

Table 1: Colony morphology for bacteria colonies from the laboratory air conditioning vents when isolated on Nutrient agar (NA) and Blood Agar (BA)

Colony	Characteristics	Colony Morphology on	
		NA	BA
1	Size	Tiny	Big
	Shape	Round	Irregular
	Elevation	Convex	Flat
	Margin	Entire	Lobate
	Opacity	Translucent	Translucent
	Surface	Shiny, smooth	Smooth
	Colour	Grey	Grey
2	Haemolytic pattern	-	Beta
	Size	Small	Small
	Shape	Round	Round
	Elevation	Flat	Flat
	Margin	Entire	Entire
	Opacity	Translucent	Translucent
	Surface	Smooth	Smooth
3	Colour	Whitish	Greyish brown
	Haemolytic pattern	-	Beta
	Size	Tiny	Small
	Shape	Round	Round
	Elevation	Raised	Flat
	Margin	Entire	Entire
	Opacity	Opaque	Translucent
4	Surface	Smooth	Smooth
	Colour	Grey	Grey
	Haemolytic pattern	-	Beta

4	Size	Big	Big
	Shape	Irregular	Irregular
	Elevation	Convex	Convex
	Margin	Lobate	Lobate
	Opacity	Translucent	Translucent
	Surface	Smooth	Smooth
	Colour	Whitish	Greyish
	Haemolytic pattern	-	Beta
5	Size	Tiny	Big
	Shape	Round	Round
	Elevation	Flat	Flat
	Margin	Entire	Entire
	Opacity	Opaque	Translucent
	Surface	Smooth	Smooth
	Colour	Grey	Grey
	Haemolytic pattern	-	Beta

Table 2: Bacteria isolated from the laboratory air conditioning vents

Colony	Isolated from				
	1	2	3	4	5
1	X	X	X	X	X
2	X	X	X	-	X
3	-	X	-	-	-
4	-	X	-	-	-
5	-	-	-	-	-

(X)–isolated; (-) -not isolated
; 1-Cytology; 2-Hematology, 3-Microbiology; 4-Molecular; 5-Parasitology

Gram stain was used to identify the five bacteria isolates and were observed under light microscope (100X magnification). All organisms in this study appeared to be gram positive with various shapes as tabulated in Table 3.

Table 3: Gram stain results for each type of bacterial colony isolated from the laboratory air conditioning vents

Colony	Gram stain	Morphology
1	+	bacilli in chain
2	+	rod-shaped in single
3	+	cocci in cluster
4	+	rod-shaped in single
5	+	bacilli in chain

(+) - Gram positive

The existence of endospores in bacterial vegetative cells was verified using spore stain. The procedure was solely for the two gram-positive bacilli (Colony 1 and 5) and two rod-shaped bacterial isolates (Colony 2 and 4). Table 4 exhibits the spore staining result for the bacteria tested.

Table 4: Spore stain results for each type of bacterial colony isolated from the laboratory air conditioning vents

Colonies	Spore stain result
1	+
2	-
4	-
5	+

(+) = presence of endospore; (-) = absence of endospore

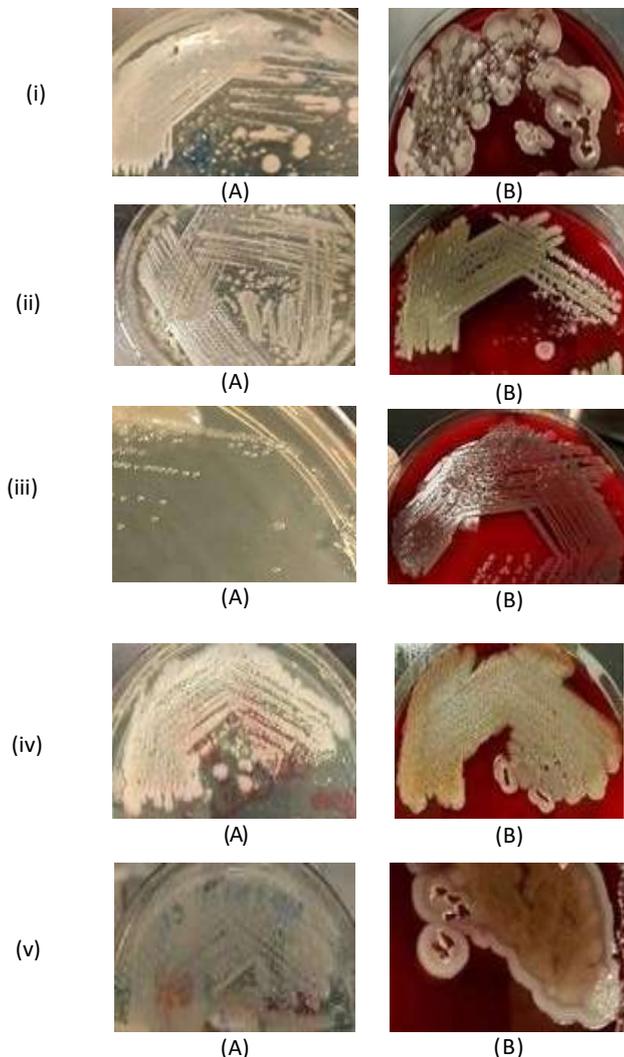


Figure 1: Colony morphology for bacteria isolates on A: Nutrient agar (NA); B: Blood Agar (BA). (i) - Colony 1; (ii) - Colony 2; (iii) - Colony 3, (iv) - Colony 4; (v) - Colony 5

Table 5: Biochemical test results of each type of bacterial colony isolated from the air conditioning vents.

Colonies	Catalase	Oxidase	Coagulase	Citrate	Indole	Mobility	TSI BUTT/Slant, Gas
1	+	-		-	-	+	K/A, no gas
2	+	+		-	-	-	K/A, no gas
3	+		-				
4	-	-		-	-	+	A/A, no gas
5	+	-		-	+	+	A/A, no gas

Note: (A) = Acid, (K) = Alkaline, (+) = Positive, (-) = Negative

Biochemical tests, such as citrate, hydrogen sulphide (H₂S), indole, motility, triple sugar iron (TSI), urea, methyl red (MR), and voges proskauer (VP) were used to further identify the bacterial isolates. The results of the biochemical tests are as stated in the table above (Table 5).

Antimicrobial sensitivity testing (AST) was then conducted on colony 2 to determine whether it is from the coagulase-negative *Staphylococcus* (CoNS) species. If the colonies were sensitive to Novobiocin, they are *Staphylococcus epidermidis*, but on the other hand, resistance to Novobiocin will indicate *Staphylococcus saprophyticus*. In the present study, colony 2 was found to be Novobiocin sensitive as shown in Table 6. Hence, colony 2 was identified as *Staphylococcus epidermidis*.

Table 6: Antimicrobial sensitivity test result for Colony 2

Indicator of zone of inhibition			
R	I	S	Result
NB30	18-21	≥22	22
≤17			(S)

(R) Resistant, (I) Intermediate, (S) Sensitive

In the present study, five bacterial genera were identified from a total of twenty-three colonies. Table 7 presents the types of bacteria isolated from the respective laboratories, while Figure 2 illustrates the prevalence of each bacterial species.

Table 7: The types of bacterial isolated from air conditioning vents according to the five different laboratories

Laboratory	Species of bacteria
Cytology	<i>Bacillus</i> spp. <i>Listeria</i> spp.
Haematology	<i>Bacillus</i> spp. <i>Listeria</i> spp. <i>Staphylococcus epidermidis</i> <i>Clostridium</i> spp.

Parasitology

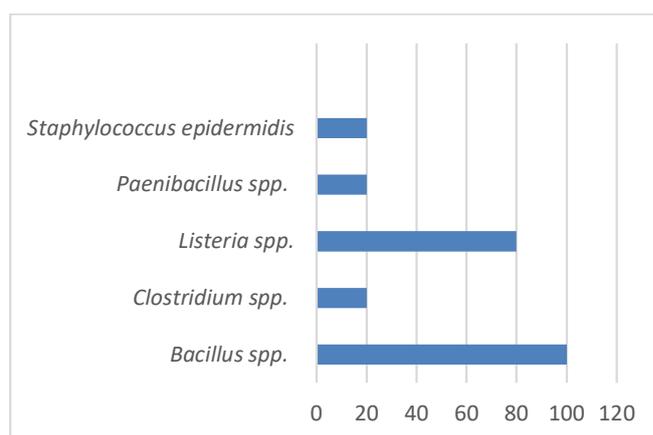
Bacillus spp.
Listeria spp.
Paenibacillus spp.

Microbiology

Bacillus spp.
Listeria spp.

Molecular

Bacillus spp.

**Figure 2:** Population of bacterial isolates from five laboratories (%) in the Centre for Medical Laboratory Technology

DISCUSSION

The spread plate method was used to isolate bacteria from the five selected laboratories which were Cytology, Haematology, Microbiology, Molecular, and Parasitology laboratories. Initially, a total of twenty-three bacterial colonies were successfully isolated and were later identified as *Bacillus* spp., *Listeria* spp., *Clostridium* spp., *Staphylococcus epidermidis* and *Paenibacillus* spp. Based on Table 7, *Bacillus* spp. was isolated from all five laboratories' air conditioning systems while *Listeria* species were obtained from four laboratories excluding the Molecular laboratory. As for *Clostridium* spp. and *Staphylococcus epidermidis*, they were only isolated from the Haematology laboratory. However, in the Parasitology laboratory, *Paenibacillus* spp. have been identified. In

addition, as shown in Figure 7, *Bacillus* spp. was the most common bacteria isolated from the five laboratories (100%), followed by *Listeria* spp. (80%). *Staphylococcus epidermidis*, *Paenibacillus* spp., and *Clostridium* spp. which were isolated from one laboratory each (20%).

Bacillus species are frequently found in dust, soil, and air in the natural environment (Sonmez et al., 2011). Most of them are normal flora in both humans and animals, except for those who are immunocompromised. Among them, *Bacillus anthracis* is particularly notable, as it can cause anthrax, a serious illness primarily transmitted through inhalation (Czekaj et al., 2024). The isolation of *Bacillus* spp. in the present study is consistent with a related study carried out by Gołofit-Szymczak and Górný (2010). Their study found that the most common bacterial species in indoor air conditioning systems were endospore-forming gram-positive rods from the genus *Bacilli*. Another study conducted in Brazil revealed that gram positive bacilli were prevalent in some areas of air conditioning units (Ross et al., 2004). However, study conducted by Sheik et al. (2015) detected coagulase-negative *Staphylococcus* (CoNS) species as the most common organism in laboratories' air conditioning vents which accounted for 26% while *Bacillus* spp. was the least common organism, 17% of the samples. On the other hand, their findings also demonstrated the presence of *Staphylococcus epidermidis*, similar to that of our study. This is further supported by studies conducted by Kumar et al. (2022) and Ortega-Peña et al. (2022) in which they found *Bacillus* spp. and *Staphylococcus epidermidis* in air conditioning systems. These two bacteria have been linked to respiratory diseases, especially involving lower and upper respiratory tract infections. Additionally, Kumar et al. (2022) mentioned that digestive disorders can also result from infection by *Bacilli*.

Paenibacillus species have been isolated from numerous sources including soil, fresh and salt water, sewage, sediments, caves, humus, compost, rhizosphere, food, plants, insect larvae and clinical samples (Sáez-Nieto et al., 2017). According to the author, they are classified as rod shaped positive or Gram-variable endospore forming aerobic or facultatively anaerobic bacteria. Ash et al. (1993) mentioned that *Paenibacillus* is also part of *Bacillus* genus. According to a study conducted in 2016 by Anas et al., spore-forming organisms such as *Bacillus* and *Paenibacillus* species can infect air conditioning system filters. A study found that dust accumulation on air conditioning filters was a common source of *Paenibacillus glucanolyticus* isolates (Osman et al., 2018). Nevertheless, Sáez-Nieto et al. (2017) noted that *Paenibacillus* species are generally regarded as contaminants, as there is limited

literature on the prevalence and clinical significance of these microorganisms.

To date, there has been no report on the presence of *Listeria* spp. and *Clostridium* spp. from air conditioner systems. However, their isolation in this study is not surprising as *Clostridium botulinum* has been isolated from laboratory staffs in Germany by Samul et al. (2013). They stated that inhalation of BoNT A from *Clostridium botulinum* poses the greatest risk to human health. In addition, Pilote et al. (2019) reported that there was evidence on the existence of *Listeria* spp. such as *Listeria monocytogenes* in indoor environments. Anas et al. (2016) have suggested that air conditioning system filters may be contaminated during repair with non-spore-producing organisms such as *Listeria* spp. and *Clostridium* spp. by human aerosols generated from activities like coughing, sneezing, and talking.

There are various internal and external factors that influence the growth of microorganisms in air conditioning systems. A study by Zhai et al. (2018) found that the diversity and quantity of airborne bioparticles are influenced by weather conditions, which will affect the formation, release, dispersion, and deposition of bacteria. According to various reports (Almaguer et al., 2014; Smets et al., 2016), temperature has a significant positive correlation with bacteria, especially bacteria that can survive at high temperatures like *Bacillus subtilis* (Kovács, 2019). Another factor that influences the growth of microorganisms is irregular cleaning of air conditioning systems. A study by Rahman (2019) emphasized the importance of regularly cleaning air conditioning systems to prevent the accumulation of heavy dust, which can promote bacterial growth. In addition, cleaning and replacing the filter can enhance the system's efficiency in preventing excessive dust buildup. Depending on factors such as the system's age, usage, history, and performance, routine maintenance of the cooling coil should be conducted two to four times a year. As humidity and temperature are known to affect bacterial viability, Al Shahwani (2005) recommended that air conditioner levels should be maintained between 20% and 60% and 20.0–24.5°C, respectively. Gołofit-Szymczak et al. (2019) suggested that aerosol disinfectant need to be sprayed and high frequency ultrasound (1.7 Hz) was employed to disinfect air conditioning system inlet and outlet ducts in order to remove microbiological contaminants. As a result, the concentration of microbial drops by more than 70%, demonstrating that microbiological contaminants can be removed by spraying disinfectant (Gołofit-Szymczak et al., 2019).

CONCLUSION

Five bacterial genera were identified in air conditioning systems across the five laboratories involved in this study: *Bacillus* spp., *Staphylococcus epidermidis*, *Clostridium* spp., *Paenibacillus* spp., and *Listeria* spp. Among them, *Bacillus* spp. was the most prevalent, followed by *Listeria* spp., *Staphylococcus epidermidis*, *Clostridium* spp., and *Paenibacillus* spp. However, as this study relied on basic microbiological tests, future research should incorporate molecular characterization techniques for more precise identification of bacterial isolates.

ACKNOWLEDGEMENT

This research was funded by the Faculty of Health Sciences, UiTM Selangor, Puncak Alam Campus.

REFERENCES

- Al Shahwani, M. F. (2005). Bacterial distribution analysis of the atmosphere of two hospitals in Ibb, Yemen. *EMHJ-Eastern Mediterranean Health Journal*, 11 (5-6), 1115-1119, 2005.
- Alangaden, G. J. (2011). Nosocomial fungal infections: epidemiology, infection control, and prevention. *Infectious Disease Clinics*, 25(1), 201-225.
- Almaguer, M., Aira, M. J., Rodríguez-Rajo, F. J., & Rojas, T. I. (2014). Temporal dynamics of airborne fungi in Havana (Cuba) during dry and rainy seasons: influence of meteorological parameters. *International Journal of Biometeorology*, 58, 1459-1470.
- Anas, G., Aligbe, D. S., Suleiman, G., & Warodi, F. A. (2016). Studies on microorganisms associated with air-conditioned environments. *IOSR J Environ Sci Toxicol Food Technol*, 10(7), 16-18.
- Ash, C., Priest, F. G., & Collins, M. D. (1993). Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test: proposal for the creation of a new genus *Paenibacillus*. *Antonie van Leeuwenhoek*, 64, 253-260.
- Ayilara, M. S., & Oginni, G. F. (2016) Bacteriological Assessment of Indoor Air-Conditioner and Grills in Academic Institutions in South Western Nigeria.
- Chan, L. L., Mak, J. W., Low, Y. T., Koh, T. T., Ithoi, I., & Mohamed, S. M. (2011). Isolation and characterization of *Acanthamoeba* spp. from air-conditioners in Kuala Lumpur, Malaysia. *Acta tropica*, 117(1), 23-30.
- Czekaj, Z., Klimowicz-Bodys, M. D., Kaniak, R., Florek, M., & Rypuła, K. (2024). *Bacillus anthracis* infection in humans and animals. *Med. Weter*, 80(7), 301-312.
- Gołofit-Szymczak, M., & Górny, R. L. (2010). Bacterial and fungal aerosols in air-conditioned office buildings in Warsaw, Poland—the winter season. *International Journal of Occupational Safety and Ergonomics*, 16(4), 465-476.
- Gołofit-Szymczak, M., Stobnicka-Kupiec, A., & Górny, R. L. (2019). Impact of air-conditioning system disinfection on microbial contamination of passenger cars. *Air Quality, Atmosphere & Health*, 12, 1127-1135.
- Govindasamy, G., Husin, U. A., Syukriani, Y. F., Sudigdoadi, S., & Mulyana, Y. (2014). Isolation and Identification of Pathogenic Fungi from Air Conditioners in Tutorial Rooms of the Faculty of Medicine, Universitas Padjadjaran. *Althea Medical Journal*, 1(1), 21-24.
- Kovács, Á. T. (2019). *Bacillus subtilis*. *Trends in microbiology*, 27(8), 724-725.
- Kumar, P., Singh, A. B., & Singh, R. (2022). Comprehensive health risk assessment of microbial indoor air quality in microenvironments. *Plos one*, 17(2), e0264226.
- Ortega-Peña, S., Rodríguez-Martínez, S., Cancino-Díaz, M. E., & Cancino-Díaz, J. C. (2022). *Staphylococcus epidermidis* controls opportunistic pathogens in the nose, could it help to regulate SARS-CoV-2 (COVID-19) infection?. *Life*, 12(3), 341.
- Osman, M. E., Ibrahim, H. Y., Yousef, F. A., Elnasr, A. A., Saeed, Y., & Hameed, A. A. (2018). A study on microbiological contamination on air quality in hospitals in Egypt. *Indoor and Built Environment*, 27(7), 953-968.
- Pilote, J., Létourneau, V., Girard, M., & Duchaine, C. (2019). Quantification of airborne dust, endotoxins, human pathogens and antibiotic and metal resistance genes in Eastern Canadian swine confinement buildings. *Aerobiologia*, 35, 283-296.
- Rahman, S. (2019). Detection of bacterial population in air conditioner and determine the ability to produce biofilm. *Iraqi Journal of Science*, 432-437.
- Ross, C., Menezes, J. R. D., Svidzinski, T. I. E., Albino, U., & Andrade, G. (2004). Studies on fungal and bacterial

population of air-conditioned environments. *Brazilian Archives of Biology and Technology*, 47, 827-835.

Sáez-Nieto, J. A., Medina-Pascual, M. J., Carrasco, G., Garrido, N., Fernandez Torres, M. A., Villalón, P., & Valdezate, S. (2017). *Paenibacillus* spp. isolated from human and environmental samples in Spain: detection of 11 new species. *New microbes and new infections*, 19, 19-27.

Samul, D., Worsztynowicz, P., Leja, K., & Grajek, W. (2013). Beneficial and harmful roles of bacteria from the *Clostridium* genus. *Acta Biochimica Polonica*, 60(4), 515-521.

Sheik, G. B., Rheam, A. I., Shehri, Z. S., & Otaibi, O. B. M. (2015). Assessment of bacteria and fungi in air from College of Applied Medical Sciences (Male) at AD-Dawadmi, Saudi Arabia. *Int Res J Biol Sci*, 4(9), 48-53.

Smets, W., Moretti, S., Denys, S., & Lebeer, S. (2016). Airborne bacteria in the atmosphere: presence, purpose, and potential. *Atmospheric Environment*, 139, 214-221.

Sonmez, E., Ozdemir, H. M., Cem, E. M., Sonmez, Y., Salacin, S., Ismail, O. C., & Sen, F. (2011). Microbiological detection of bacteria and fungi in the autopsy room. *Rom J Leg Med*, 19(1), 33-44.

Wise, K. (2006). Preparing spread plates protocols. *Am. Soc. Microbiol. Microbe Libr.* Available at: <http://www.asmscience.org/content/education/protocol/protocol,3085>.

Wopereis, D. B., Bazzo, M. L., de Macedo, J. P., Casara, F., Golfeto, L., Venancio, E., ... & Caumo, K. S. (2020). Free-living amoebae and their relationship to air quality in hospital environments: characterization of *Acanthamoeba* spp. obtained from air-conditioning systems. *Parasitology*, 147(7), 782-790.

Zhai, Y., Li, X., Wang, T., Wang, B., Li, C., & Zeng, G. (2018). A review on airborne microorganisms in particulate matters: Composition, characteristics and influence factors. *Environment international*, 113, 74-90.

Education for Improving Awareness and Practices Regarding Hand Hygiene Among Secondary School Students

Mohd Nazri Abu^{1,3}, Mohd Fahmi Mastuki^{1,3}, Wan Shahrman Yushdie Wan Yusoff¹, Farah Iwanina Yusoff¹, Norazah Abu Bakar² and Wan Mazlina Md Saad^{1,3}

¹Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

²Sekolah Menengah Kebangsaan Meru, Batu 7, Pekan Meru 41050 Klang Selangor.

³Research and Education in Biomedical, Epidemiology and Immunology Aliances (REBEIA), FSK, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Handwashing is a fundamental practice for preventing the transmission of communicable diseases such as diarrhoea, pneumonia, and foodborne infections, especially in educational environments. Despite its significance, poor handwashing practices remain prevalent among secondary school students, often due to inadequate facilities and limited hygiene education. This study aimed to describe the implementation, effect and process evaluation of an educational session focused on promoting hand hygiene among the secondary school children. A structured questionnaire was administered to 40 selected canteen prefects to assess their handwashing knowledge and practices before and three weeks after a demonstration of proper handwashing techniques. Following the demonstration, a group of prefects instructed non-prefect students in handwashing, and both groups were observed for compliance. Statistical analysis revealed a significant improvement in knowledge and routine among the canteen prefects after the demonstration, as indicated by the paired t-test. The Mann-Whitney test further demonstrated that canteen prefects outperformed non-prefect students in terms of knowledge, routine, and adherence to proper handwashing steps. These findings suggest that focused educational programs can significantly enhance hand hygiene knowledge and practices. The results support the development of initiatives aimed at improving handwashing behaviours to prevent infectious diseases, particularly within school environments.

Keywords:

hand hygiene; secondary school students; canteen prefects

INTRODUCTION

Handwashing is a fundamental public health practice that involves using water, with or without soap, to clean the hands and remove dirt, pathogens, and microbes. It is crucial in preventing the transmission of infectious diseases, especially after physical encounters in public spaces. During the coronavirus disease 2019 (COVID-19) pandemic, the World Health Organization (WHO) emphasized handwashing as a vital public health intervention to curb the spread of the SARS-CoV-2 virus (Labana, 2024). In everyday settings, poor hand hygiene contributes to the spread of diseases such as diarrhoea and pneumonia, particularly after activities such as using the bathroom, coughing, blowing the nose, playing with pets, and touching contaminated surfaces (Mbakaya, 2019; Dutta et al., 2020; Melese et al., 2019; Chittleborough et al., 2012). Handwashing is especially effective in controlling the transmission of foodborne and faecal-oral pathogens, with numerous infectious diseases being spread via contaminated hands.

Journal homepage: <https://journals.iium.edu.my/ijahs/index.php/IJAHs>
EISSN NO 2600-8491

Bacteria and viruses, such as *Salmonella* spp., *Escherichia coli* O157, norovirus, and adenovirus, are common culprits in gastrointestinal and respiratory illnesses. These pathogens are transmitted through contaminated objects or direct contact, which underscores the importance of hand hygiene (Enkhbat et al., 2022). Simple and inexpensive, handwashing with soap has proven to be one of the most cost-effective methods for reducing the burden of infectious diseases globally (Enkhbat et al., 2022). Numerous studies have demonstrated that effective handwashing with antiseptic cleansers or hand rubs can significantly reduce the risk of infection. According to the Centres for Disease Control and Prevention (CDC), regular handwashing with soap and water can decrease the incidence of diarrheal disease-related deaths by up to 50% (CDC, 2020). Research has also suggested that a 10 to 15-second handwashing duration is generally sufficient to remove transient bacteria from hands (Jemal, 2018). However, poor handwashing habits are still prevalent in many populations, often due to insufficient awareness or education. Nicolaidis et al.

* Corresponding author.
E-mail address: nazri669@uitm.edu.my

(2019) noted that behavioural changes regarding hand hygiene are challenging, particularly in communities with limited access to resources or where hygiene practices are not integrated into daily routines. Research in Ethiopia found that factors such as hygiene education, access to sanitation facilities, and parental health education significantly influenced hand hygiene behaviour (Assefa & Kumie, 2014). These findings underscore the importance of targeted education and promotion campaigns to enhance hand hygiene practices. Several factors, such as the unavailability of soap dispensers or hand dryers, contribute to poor hand hygiene, particularly in schools where children are most vulnerable to infections. Ngimbwa et al., 2020) identified that inadequate infrastructure in schools such as empty soap dispensers and lack of drying facilities hinders proper hand hygiene practices. Schools play an essential role in teaching hygiene and health habits, providing a setting where children can develop lifelong skills. In developing countries, children often lack basic handwashing skills, making it critical for schools to incorporate hygiene education into their curriculum (Otsuka et al., 2019).

A study conducted in the Klang Valley region found that only 63% of preschool children demonstrated good handwashing practices (Jamaluddin et al., 2020). Given the importance of hand hygiene in preventing infections, this study aims to explore the handwashing knowledge and practices among lower-form secondary school students in Malaysia. The study will examine how well students understand the importance of hand hygiene and their ability to apply this knowledge in daily life. Canteen prefects in secondary schools play an essential role in maintaining the cleanliness and organization of the school canteen. Their tasks typically include overseeing food distribution, ensuring that the canteen remains hygienic, and managing students' behaviour during mealtime. One of the most critical responsibilities they hold is monitoring food safety practices, which includes ensuring proper hand hygiene among both canteen staff and students and reporting to the schoolteacher if any disobedience is detected among canteen operators.

Hand hygiene is vital in preventing the spread of germs and foodborne illnesses. Canteen prefects must be aware of the correct handwashing techniques, including washing hands with soap and water for at least 20 seconds, especially before handling food, after touching potentially contaminated surfaces, and after using the restroom. They should also promote the importance of using hand sanitisers when soap and water aren't available and regularly remind their peers and staff of the role hand hygiene plays in maintaining a safe eating environment. This research is significant as it will provide insights into

the current state of handwashing knowledge and practices among secondary school students in Malaysia. These findings could serve as the basis for designing educational programs and materials to enhance hand hygiene awareness and behaviour. Furthermore, the results of this study may offer valuable data for the Malaysian Ministry of Health (MOH) and the Ministry of Education in revising school health education policies to better address hygiene and health promotion. By raising awareness of proper handwashing, this study will contribute to improving public health outcomes, reducing the incidence of infectious diseases, and fostering healthier behaviours among young people. To assess handwashing knowledge and practice among secondary school students in Malaysia, this study aims to evaluate lower-form students using a structured questionnaire. It will compare first-year and second-year students' levels of knowledge, identify influencing factors, and propose recommendations to enhance hygiene education and handwashing practices within schools.

MATERIALS AND METHODS

Study design

This study utilized a cross-sectional research design targeting students aged 13 to 15 years from selected secondary schools in Klang, Selangor. A total of 40 participants were selected, accounting for a 10% increase to adjust for potential non-responses. Consent from both students and their guardians was obtained before participation, ensuring ethical compliance. Initially, proper handwashing techniques were demonstrated to the selected canteen prefects. The study used a structured questionnaire before and after the intervention to assess the knowledge and practice of proper hand hygiene (Yekta et al., 2021; Almoslem et al., 2021). The study was conducted in two phases: before and two weeks after the handwashing demonstration. This structure allowed for an evaluation of the short-term retention of the handwashing practices among the prefects, as well as their ability to convey these skills to non-prefect students. The evaluation followed the World Health Organization's (WHO) handwashing guidelines, focusing on both knowledge acquisition and practical skills (Toney-Butler et al., 2025).

Sample selection

The sample included 40 students selected based on inclusion and exclusion criteria. Only canteen prefects from secondary school in Klang, Selangor were eligible for participation. Students who were not prefects or from unselected schools were excluded. These criteria ensured

a targeted focus on individuals responsible for promoting hygiene in the school's food-serving environment as described in the previous study (Ismail et al., 2024).

Sample size calculation

Using Yamane's formula, the sample size was calculated as follows:

$$n = \frac{N}{1 + N(e)^2}$$

Where: n = sample size, N = total population size and e = margin of error (expressed as a decimal). To account for potential non-response, a 10% buffer was added, making the final sample size 40 participants. This calculation ensures a representative sample with a 5% margin of error and a 95% confidence level, adequate for assessing handwashing knowledge and practice (Chavda et al., 2020).

Study tools

The primary data collection tool was a structured questionnaire based on previously validated studies (Almoslem et al., 2021; (Ismail et al., 2024). The questionnaire had 11 items divided into three sections:

- a. Demographics: Information on age, gender, and other background factors.
- b. Handwashing Routine: Frequency and techniques used in handwashing.
- c. Knowledge: Evaluation of the student's understanding of correct handwashing techniques, as per WHO guidelines.

The structured questionnaire format ensured that both qualitative and quantitative data were collected, with pre- and post-intervention comparisons offering insights into the effectiveness of handwashing education (Rahman et al., 2021).

Ethical consideration

Ethical approval was obtained from the Research Ethics Committee of Universiti Teknologi MARA (UiTM) [Reference no: FERC/FSK/MR/2023/00150] before the study commenced. Participants were informed of their rights, including the voluntary nature of participation and their ability to withdraw at any point without consequences. Confidentiality and data security were prioritized to protect participants' identities and responses.

Data collection process

Data were collected in two phases:

Phase 1: A pre-intervention questionnaire was administered to canteen prefects. Afterwards, a handwashing demonstration was conducted, following WHO guidelines. The canteen prefects were evaluated based on their ability to follow the demonstration.

Phase 2: Two weeks later, the prefects completed a post-intervention questionnaire to assess retention of the handwashing practices. Additionally, the prefects demonstrated the proper handwashing technique to five non-prefect students, who were then evaluated on their adherence to the demonstrated techniques. This method allowed for both direct and peer evaluation, adding depth to the assessment (Yekta et al., 2021).

Data analysis

SPSS version 28 was used for data analysis. Descriptive statistics were employed to summarize the demographic information and handwashing practices. An independent t-test was used to compare knowledge and practices before and after the intervention. A paired t-test was conducted to measure the effectiveness of the handwashing demonstration in changing behaviours (Chavda et al., 2020). Categorical variables (such as gender) were analysed using frequency distributions, while continuous variables were summarized with means and standard deviations.

RESULTS

Demographic data of Canteen Prefect

Table 1 - Demographic data of canteen prefects.

Data	Numbers and percentage
Number of Subjects	40 (100%)
Gender	
Male	22 (55%)
Female	18 (45%)
Age	
13	0 (0%)
14	15 (37.5%)
15	10 (25%)
16	7 (17.5%)
17	8 (20%)

The demographic data of the respondents, the canteen prefects of SMK Meru, shows that a total of 40 prefects participated in this survey, selected randomly. Most of the respondents were male (55%), with the remaining 45% female. Their ages ranged from 14 to 17 years old. The largest group of respondents (15) were 14 years old,

followed by 10 respondents aged 15, 8 aged 17, and 7 aged 16. There were no respondents aged 13.

Table 2 - Comparison of handwashing routine and handwashing knowledge before and after watching handwashing demonstration video using paired t-test.

Variable	Before Demonstration Mean (SD)	After Demonstration Mean (SD)	Mean Difference (95% CI)	t-stats (df)	P-value
Routine	15.30 (3.20)	17.88 (1.64)	2.58 (1.48, 3.66)	4.76 (39)	<0.001
Knowledge	8.60 (2.48)	10.07 (2.18)	1.48 (0.26, 2.69)	2.45 (39)	0.019

The comparison of handwashing routines and knowledge before and after watching a handwashing demonstration video was analysed using a paired t-test. The mean score for the handwashing routine increased from 15.30 (SD = 3.20) before the demonstration to 17.88 (SD = 1.64) afterwards, with a mean difference of 2.58 (95% CI: 1.48, 3.66). The t-statistic was 4.76 (df = 39), and the improvement was statistically significant with a P-value of <0.001. Similarly, the mean score for handwashing knowledge improved from 8.60 (SD = 2.48) before the demonstration to 10.07 (SD = 2.18) afterwards, showing an average difference of 1.48 (95% CI: 0.26, 2.69). The t-statistic for this comparison was 2.45 (df = 39), with a significant P-value of 0.019. These results demonstrate a significant positive impact of the handwashing demonstration video on both routine and knowledge.

Table 3 - comparison of handwashing routine, knowledge and steps performed between the canteen prefects and non-prefect students using Mann Whitney test.

Variable	Canteen Prefects (n=5) Median (IQR)	Non-Prefect Students (n=5) Median (IQR)	Z statistic	P value
Routine	18 (3)	15 (2)	-2.48	0.016
Knowledge	12 (0)	7 (1.5)	-2.81	0.008
Handwashing steps	10 (0.5)	8 (2.5)	-1.54	0.222

Comparing the handwashing routine, knowledge, and steps performed between canteen prefects and non-prefect students using the Mann-Whitney test. Canteen prefects had a higher median and inter-quartile range (IQR) handwashing routine score of 18 (IQR = 3) compared to 15 (IQR = 2) for non-prefect students, with a Z statistic of -2.48 and a significant P-value of 0.016. Prefects also demonstrated greater knowledge, with a median of 12 (IQR = 0) compared to 7 (IQR = 1.5) for non-prefects (Z = -2.81, P = 0.008). However, the difference in handwashing steps performed was not significant, with prefects having a median of 10 (IQR = 0.5) versus 8 (IQR = 2.5) for non-prefects (Z = -1.54, P = 0.222).

DISCUSSION

This study aimed to assess the knowledge and practice of handwashing among the canteen prefects at selected schools in Klang, Selangor. The study focused on the role of hand hygiene as a critical infection control measure. Educational institutions have long been identified as vital settings for promoting hand hygiene practices, which are fundamental in preventing the spread of infectious

diseases, especially considering the COVID-19 pandemic (Mohamed et al., 2022). Adequate knowledge of handwashing techniques is strongly associated with improved hygiene practices (Tengku Jamaluddin et al., 2020; Almoslem Munthir et al., 2021).

Before viewing the demonstration video, over 50% of the canteen prefects consistently washed their hands before and after meals but only occasionally practised handwashing after coughing or blowing their nose. This mirrors findings from previous research where most students demonstrated better hygiene practices before meals and after using the restroom (Hazazi, 2018). Post-intervention, a significant increase was observed in handwashing after activities like handling garbage or when hands were visibly dirty, aligning with research by Rosenthal et al. (2013), who reported improved hygiene compliance following targeted educational interventions.

Furthermore, before the intervention, most of the canteen prefects recognized the importance of washing hands with soap and water for personal hygiene and disease

prevention, which aligns with global research emphasizing that handwashing with soap is more effective than using water alone (Pradhan & Mondal, 2020; Eshetu et al., 2020). However, only 20% strongly agreed on the importance of drying hands after washing. This low awareness reflects similar gaps identified in a study conducted by Sharma et al. (2020), where drying was often overlooked despite its importance in removing residual moisture and bacteria. Following the video demonstration, significant improvements were noted in knowledge across all hand hygiene-related aspects, including the importance of drying hands, which rose to 42.5%.

Statistical analysis using paired t-tests confirmed that the intervention had a significant impact on both routine and knowledge. For the handwashing routine, the mean difference pre- and post-intervention was statistically significant ($t(39) = 4.76, p < 0.05$), and the same was true for knowledge scores ($t(39) = 2.45, p < 0.05$). These results align with findings from similar interventions, where visual demonstrations resulted in measurable improvements in hygiene behaviour (Dangis et al., 2023).

Observations comparing canteen prefects and non-prefect students revealed that the prefects performed handwashing steps more thoroughly, adhering closely to WHO's recommended handwashing duration of 40-60 seconds (WHO, 2020). This observation aligns with Toney-Butler (2023), who highlighted the importance of proper handwashing duration for maximum germ removal. However, the non-prefects were less consistent in performing steps like interlacing fingers or rubbing thumbs, as 20% of them failed to meet the time standard for proper handwashing, consistent with Jamaluddin et al. (2020), who found that students often skip critical steps such as rubbing the backs of their hands.

The Mann-Whitney test demonstrated statistically significant differences between canteen prefects and non-prefect students in terms of handwashing routine ($z = -2.48, p = 0.016$) and knowledge ($z = -2.81, p = 0.008$), while differences in handwashing steps were not significant ($z = -1.54, p = 0.222$). These findings indicate that while canteen prefects demonstrated better overall hand hygiene practices, further interventions are needed to ensure full adherence to recommended techniques across all students.

The study faced several limitations, including reliance on online sessions due to extremely hot weather (40C) and pandemic constraints, which may have impacted the depth of in-person engagement. Conducting the study at a single school limits the generalizability of the findings, and the two-week follow-up may not capture long-term

retention of handwashing practices. Additionally, self-reported data could introduce bias, as students might over-report positive behaviours. Future research should include a larger, more diverse sample and a longer follow-up period to assess lasting behaviour changes.

CONCLUSION

This study provided valuable insights into the knowledge and practice of handwashing among the canteen prefects at SMK Meru. The findings demonstrate the effectiveness of using educational videos to enhance hand hygiene awareness and adherence to recommended practices. Notably, the canteen prefects displayed significant improvements in both knowledge and practice following the intervention, with knowledge transfer extending to non-prefect students. These results highlight the potential for peer-led hygiene programs to serve as effective tools for promoting hand hygiene in schools. Future research should explore the long-term effects of such interventions and investigate other factors, such as the availability of handwashing facilities, that may influence hand hygiene practices. Additionally, continued efforts to educate students about the importance of drying hands and completing all handwashing steps will further reduce the risk of infection transmission in school settings.

ACKNOWLEDGEMENT

The authors are especially appreciative to Canteen Prefect Unit, SMK Meru, Klang for participating actively in the research. In addition, the authors would like to Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, UiTM Selangor Branch, Puncak Alam for enabling the research to be conducted and for offering comprehensive services, resources, and conducive work environment.

REFERENCES

- Almoslem, M. M., Alshehri, T. A., Althumairi, A. A., Aljassim, M. T., Hassan, M. E., & Berekaa, M. M. (2021). Handwashing Knowledge, Attitudes, and Practices Among Students in Eastern Province Schools, Saudi Arabia. *Journal of Environmental and Public Health*, 2021, 6638443. <https://doi.org/10.1155/2021/6638443>
- Assefa, M., & Kumie, A. (2014). Assessment Of Factors Influencing Hygiene Behaviour Among School Children in Mereb-Leke District, Northern Ethiopia: A Cross-Sectional Study. *BMC Public Health*, 14(1). <https://doi.org/10.1186/1471-2458-14-1000>

- CDC (2020). Handwashing: Clean hands save lives. Centers for Disease Control and Prevention. Retrieved from <https://www.cdc.gov>
- (2022) Therapeutics to Tackle Omicron Outbreak. *Immunotherapy* 14:11, pages 833-838.
- Chittleborough, C. R., Nicholson, A., Basker, E., Bell, S., & Campbell, R. (2012). "Factors Influencing Hand Washing Behaviour in Primary Schools: Process Evaluation Within a Randomized Controlled Trial." ("Improving young children's handwashing behaviour and understanding of ...") *Health Education Research*, 27(6), 1055-1068. <https://doi.org/10.1093/her/cys061>
- Dangis, G., Terho, K., Graichen, J., Günther, S. A., Mieronkoski, R., Salanterä, & Pakarinen, A. (2023). ("A framework for designing hand hygiene educational interventions in ...") *Hand Hygiene of Kindergarten Children—Understanding the Effect of Live Feedback on Handwashing Behaviour, Self-Efficacy, And Motivation of Young Children: Protocol for A Multi-Arm Cluster Randomized Controlled Trial.* *Plos One*, 18(1), e0280686. <https://doi.org/10.1371/journal.pone.0280686>
- Dutta, G., T, S., & Kumar, T. (2020). "Knowledge And Practice of Hand Hygiene Among Undergraduate Students and Junior Doctors in The Regional Institute of Medical Sciences." ("Review of literature: Knowledge and practice of ... - ScienceDirect") *Journal of Family Medicine and Primary Care*, 9(9), 4741. https://doi.org/10.4103/jfmpc.jfmpc_794_20
- Enkhbat, M., Togoobaatar, G., Erdenee, O., & Katsumata, A. T. (2022). Handwashing Practice among Elementary Schoolchildren in Urban Setting, Mongolia: A School-Based Cross-Sectional Survey. *Journal of environmental and public health*, 2022, 3103241. <https://doi.org/10.1155/2022/3103241>
- Eshetu, D., Kifle, T., & Hirigo, A. T. (2020). Knowledge, Attitudes, and Practices of Hand Washing Among Aderash Primary Schoolchildren in Yirgalem Town, Southern Ethiopia. *Journal of Multidisciplinary Healthcare*, Volume 13, 759-768. <https://doi.org/10.2147/jmdh.s257034>
- Haque, M. (2020). Handwashing In Averting Infectious Diseases: Relevance to Covid-19. *Journal of Population Therapeutics & Clinical Pharmacology*, 27(SP1), e37-e52.
- Chavda Vivek P, Eswara Naga Hanuma Kumar Ghali, Murali M Yallapu & Vasso Apostolopoulos. <https://doi.org/10.15586/jptcp.v27sp1.711>
- Hazazi, A. (2018). Knowledge Attitude and Practices Regarding Personal Hygiene Among the Male Primary School Children in Abha, Kingdom of Saudi Arabia: a cross-sectional study. *Helix*, 8(2), 3215-3223. <https://doi.org/10.29042/2018-3215-3223>
- Ismail, S. R., Radzi, R., Megat Kamaruddin, P. S. N., Lokman, E. F., Lim, H. Y., Abdul Rahim, N., Yow, H. Y., Arumugam, D., Ngu, A., Low, A. C. Y., Wong, E. H., Patil, S., Madhavan, P., Nordin, R. B., van der Werf, E., & Lai, N. M. (2024). "The Effects of School-Based Hygiene Intervention Programme: Systematic Review and Meta-Analysis." ("Yow Hui Yin - Google Scholar") *Plos one*, 19(10), e0308390. <https://doi.org/10.1371/journal.pone.0308390>
- Jamaluddin, T. Z. M. T., Mohamed, N. A., Rani, M. D. M., Ismail, Z., Ramli, S., Faroque, H., & Isahak, I. (2020). Assessment On Hand Hygiene Knowledge and Practices Among Pre-School Children in Klang Valley. *Global Paediatric Health*, 7. <https://doi.org/10.1177/2333794x20976369>
- Jemal, S. (2018). "Knowledge and Practices of Hand Washing among Health Professionals in Dubti Referral Hospital, Dubti, Afar, Northeast Ethiopia." ("Knowledge and Practices of Hand Washing among Health Professionals in ...") *Advances in Preventive Medicine*, 2018, 1-7. <https://doi.org/10.1155/2018/5290797>
- Mbakaya, B. C. (2019). Community-Based Interventions for Preventing Diarrhoea in People Living with HIV In Sub-Sahara Africa: A Systematic Review. *Malawi Medical Journal*, 31(1), 86. <https://doi.org/10.4314/mmj.v31i1.15>
- "Melese, B., Paulos, W., Astawesegn, F. H., & Gelgelu, T. B. (2019). ("Sci-Hub | Prevalence of diarrheal diseases and associated factors among ...") "Prevalence of Diarrheal Diseases and Associated Factors Among Under-Five Children in Dale District, Sidama Zone, Southern Ethiopia: a cross-sectional study." ("Prevalence of diarrheal diseases and associated factors among under ...") *BMC Public Health*, 19(1). <https://doi.org/10.1186/s12889-019-7579-2>

- Mohamed NA, Solehan HM, Mohd Rani MD, Ithnin M, Arujanan M (2023) Understanding COVID-19 Vaccine Hesitancy in Malaysia: Public Perception, Knowledge, And Acceptance. *Plos ONE* 18(4): e0284973. <https://doi.org/10.1371/journal.pone.0284973>
- Ngimbwa, J., Basinda, N., Kapesa, A., & Ngallaba, S. (2020). Evaluation Of the School Water, Sanitation and Hygiene National Strategic Implementation Plan (2012 -2017) In Ukerewe District, North-Western Tanzania. <https://doi.org/10.20944/preprints202008.0390.v1>
- Nicolaidis, C., Avraam, D., Cueto-Felgueroso, L., González, M. C., & Juanes, R. (2019). "Hand-Hygiene Mitigation Strategies Against Global Disease Spreading Through the Air Transportation Network." ("(PDF) Hand-hygiene mitigation strategies against global disease ...") <https://doi.org/10.1101/530618>
- Otsuka, Y., Agestika, L., Harada, H., Sriwuryandari, L., Sintawardani, N., & Yamauchi, T. (2019). Comprehensive Assessment of Handwashing and Faecal Contamination Among Elementary School Children in an Urban Slum of Indonesia. *Tropical Medicine; International Health*, 24(8), 954-961. <https://doi.org/10.1111/tmi.13279>
- Pradhan, M. R. and Mondal, S. (2020). Pattern, Predictors and Clustering of Handwashing Practices in India. *Journal of Infection Prevention*, 22(3), 102-109. <https://doi.org/10.1177/1757177420973754>
- Rosenthal, V. D., Pawar, M., Leblebicioğlu, H., Navoa-
Ng, J. A., Villamil-Gómez, W. E., Armas-Ruiz, A., & Kübler, A. (2013). Impact of the International Nosocomial Infection Control Consortium (Inicc) Multidimensional Hand Hygiene Approach Over 13 Years In 51 Cities Of 19 Limited-Resource Countries from Latin America, Asia, The Middle East, And Europe. *Infection Control; Hospital Epidemiology*, 34(4), 415-423. <https://doi.org/10.1086/669860>
- Sugita, E. W. (2022). "Water, Sanitation and Hygiene (Wash) in Japanese Elementary Schools: Current Conditions and Practices." ("Drinking water, sanitation, and hygiene (WASH) situation in primary ...") *Paediatrics International*, 64(1). <https://doi.org/10.1111/ped.15062>
- Toney-Butler TJ, Gasner A, Carver N. Hand Hygiene. [Updated 2023 Jul 31]. In: Stat Pearls [Internet]. Treasure Island (FL): Stat Pearls Publishing; 2025 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK470254/>
- V. Labana, R., E. Cruz, M. K., M. Ramirez, A. A. J., & E. Raya, M. (2024). Did Covid-19 Risk Perceptions Influence the Handwashing Behaviour of the Filipino Population. *International Journal of Public Health Science (IJPHS)*, 13(3), 1102. <https://doi.org/10.11591/ijphs.v13i3.23993>
- Yekta Reza, Leily Vahid-Dastjerdi, Sahar Norouzbeigi, Amir M. Mortazavian. (2021). Food Products as Potential Carriers of SARS-Cov-2, *Food Control*, Volume 123, 107754, ISSN 0956-7135, <https://doi.org/10.1016/j.foodcont.2020.107754>.

Impact of Virtual Imaging Tools on Diagnostic Accuracy in Respiratory Cytology Education

Nuraishah Mohamad¹, Wan Shahrman Yushdie Wan Yusoff¹, Mohd Nazri Abu^{1,2*}

¹Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, UiTM Selangor Branch, 42300 Puncak Alam, Selangor, Malaysia

²Research and Education in Biomedical, Epidemiology and Immunology Aliances (REBEIA), FSK, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Background: In a standard cytology laboratory setting, cells derived from either fine needle aspiration cytology (FNAC) or body fluids are examined under the microscope to detect early pathophysiological states. Recent technological advancements have created a virtual imaging tool such as whole slide imaging (WSI), which enables the conversion of cytology and histology glass slides to digital form, allowing pathologists to review them on internet-connected devices. However, due to budgetary constraints in acquiring the software and hardware necessary for scanning glass slides and converting them into digital images, there is a concerted effort to adopt digital online platforms as a cost-effective solution. Despite these technological advancements, the current pedagogical approach relies significantly on hands-on laboratory training. In this study, we implemented a method of digitising microscopic views through an online digital platform as a virtual digital tool to instruct students in diagnosing respiratory cytology cases. Our objective is to evaluate the effectiveness of this learning mode and its impact on students' practical skills by assessing their diagnostic accuracy. **Methods:** Five students participating in the digital online learning modality were evaluated across two separate sessions, each involving a review of thirty cytology glass slide cases. The diagnoses obtained were then converted into data to be statistically analysed using SPSS software. **Results:** The results indicated inter-observer reliability, assessed using Fleiss' kappa (κ), was 0.468 in the first session and improved to 0.506 in the second session. Meanwhile, Cohen's kappa value, which evaluates the intra-observer reliability, shows a moderate agreement ($\kappa=0.557$). Additionally, the evaluation results indicated an average sensitivity of 86.68%, a specificity of 80.38%, a positive predictive value (PPV) of 34.22%, a negative predictive value (NPV) of 97.9%, a likelihood ratio (LR) of 5.583, and an overall diagnostic accuracy of 80.33%. **Conclusion:** In conclusion, even though digital images offer valuable theoretical knowledge, practical microscopy experience is essential for accurate diagnoses in respiratory cytology

Keywords:

cytology; diagnostic accuracy; digital imaging; reliability test; respiratory cytology

INTRODUCTION

In the current standard cytology laboratory practice, the samples are processed into cytological glass slides and examined under the microscope for detection of pathophysiological states. In recent years, extensive research has focused on advancing computational technologies within the healthcare system to enhance the accuracy and efficiency of medical diagnostics and patient care. A notable development in this field includes introducing whole slide imaging (WSI) systems in cytopathology, which enable the digitisation of histological and cytological glass slides (Kumar et al., 2020). These systems effectively replicate the traditional microscopic examination of pathology slides digitally, offering

significant potential for improved diagnostic workflows (Zarella et al., 2019). Besides facilitating primary diagnosis, the WSI system enables efficient sharing of slide cases, making it valuable for various applications such as training, research, and teleconsultation. These advantages have contributed to the system's growing popularity within academic settings in recent decades (Eccher & Girolami, 2020).

The current conventional pedagogical approach in cytology classes significantly relies on the practical tutor in the laboratory. This immersive method provides hands-on experience, equipping students with the essential diagnostic skills to accurately report cytological cases. In response to the growing demand for new knowledge and

* Corresponding author.

E-mail address: nazri669@uitm.edu.my

advancements in the medical field, medical education has increasingly incorporated online learning in recent years. However, due to budgetary constraints in acquiring the software and hardware necessary for scanning glass slides and converting them into digital images, there is a concerted effort to adopt digital online platforms as a cost-effective solution.

In this study, we employed a digital imaging system using an online platform to teach respiratory cytology. Respiratory cytology is a part of cytology that examines the aspirated cells from the respiratory organ. This study was conducted using a camera-equipped microscope to project cytology slide views onto a monitor, which were then shared via Google Meet. This setup allowed students to observe live screenings, focusing, and movement of the glass slides in real time.

This method is not commonly employed in teaching the practical skills of respiratory cytology, and its impact on students' competency in reporting respiratory cytology cases remains unexplored. Therefore, this study was conducted to evaluate the diagnostic accuracy of undergraduate students trained using digital imaging in reporting respiratory cytology cases.

MATERIALS AND METHODS

Experimental pedagogical method

This study was conducted following ethical clearance granted by the Faculty Research Ethics Committee (Approval No.: FERC/FSK/MR/2022/0089). A two-hours practical session on respiratory cytology was conducted via Google Meet, where a live microscopic view was displayed on a 52-inch LCD monitor through a LEICA ICC50 digital camera-equipped LEICA DM750 compound microscope. Additionally, the high definition (HD) web camera connected to a laptop allowed remote students to participate in real time, as shown in Figure 1.

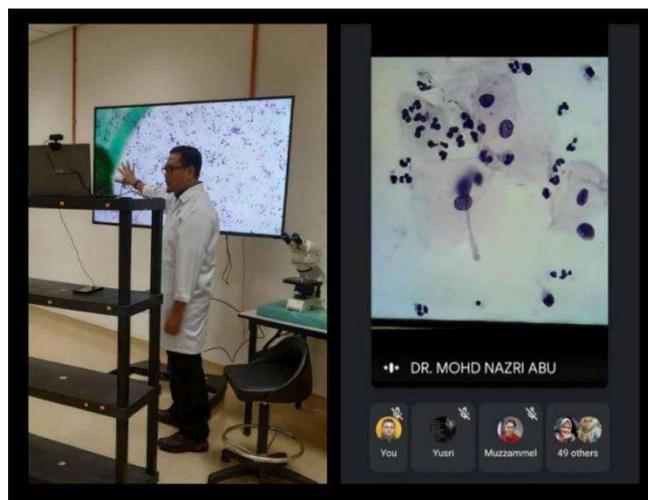


Figure 1: Teaching and learning process through online distance learning via Google Meet in cytology laboratory, UiTM.

Cases selection

A total of thirty respiratory cytology glass slides, each stained with Papanicolaou (PAP) stain, were randomly selected from the slide archive of the Cytology Laboratory at UiTM Selangor, Puncak Alam Campus. The slides, numbered from 1 to 30, were carefully chosen based on specific criteria to ensure an adequate sample collection and the absence of any artifacts. Slides with indeterminate diagnoses or any form of damage were excluded from the study.

Students' selection

Five undergraduate students from the Bachelor of Medical Laboratory Technology (MLT) program were voluntarily selected based on specific inclusion criteria, which included proficiency in operating a light microscope and successful completion of the cytology course. Students were excluded if they had not participated in the online session, had impaired color vision, or were unable to attend both screening sessions.

Screening sessions

The physical screening session was conducted in the cytology laboratory of UiTM Selangor Puncak Alam Campus. The students were given the thirty labelled slides and were instructed to diagnose them based on the following classifications: benign, atypical, or malignant. The screening session was conducted in two parts, namely Session A and Session B, to observe the data from the same students but on different occasions. These data were used for the intra-observer reliability test. Both sessions

included the same thirty respiratory cytology cases arranged randomly. Session B was conducted one month after Session A. The agreement between the diagnoses made between the students was also evaluated for an inter-observer reliability test. All of the diagnoses made by the students will also be compared to the actual diagnoses for the evaluation of diagnostic accuracy (Gupta et al., 2001).

Statistical analysis

The results were tabulated and analysed using the SPSS software (Statistical Package for Social Science, IBM Corp, New York, USA). The inter- and intra-observer reliability was measured using the kappa coefficient value for the first objective. Cohen's kappa is used to assess different parameters by the same individuals, also known as intra-observer reliability. Inter-observer reliability was assessed using Fleiss' kappa, which is the assessment of the same parameter by multiple individuals (N. Roslan et al., 2021).

The second goal was to evaluate each student's diagnostic accuracy in the respiratory cytology case based on the operating parameters. The standard statistic sets for diagnostic assessment are sensitivity, specificity, predictive values, and likelihood ratios. The method was adapted from another similar study which utilises the 2x2 contingency table; thus, the results were categorised into only 'benign' and 'malignant'. For this study, atypical cases were classified as 'malignant' (N. Roslan et al., 2022).

First, the results produced by the students were compared with the reference results, which were then tabulated into true positive (TP), true negative (TN), false positive (FP), and false negative (FN). These data will be further analysed based on the operating parameters, which are sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratio (LR). In addition, the receiver operating characteristic (ROC) curve was constructed, and the area under the curve (AUC) was measured.

RESULTS

SPSS data analysis was performed from the data obtained to measure the Fleiss' kappa, Cohen's kappa, and diagnostic accuracy of the students. Table 1 and 2 show the calculated Fleiss' kappa which was used to determine the level of agreement between the students.

Table 1: Calculated Fleiss' kappa value and the strength of agreement in both screening sessions.

Screen session	Fleiss' kappa value	Strength of agreement	Asymptomatic standard error	P value	95% Confidence Interval (CI)	
					Lower	Upper
A	0.353	Fair	0.044	<0.001	0.267	0.439
B	0.506	Moderate	0.046	0.000	0.417	0.596

*κ value <0.20 indicated as 'Poor' agreement, 0.21-0.40 as 'Fair' agreement, 0.41-0.60 as 'Moderate' agreement, 0.61-0.80 as 'Good' agreement and 0.81-1.00 as 'Very good' agreement.

The level of agreement between the students was determined using Fleiss' kappa. As shown in Table 1, the calculated Fleiss kappa was interpreted as fair in the first session. The value of Fleiss' kappa for the second session, on the other hand, was between 0.41 and 0.60; thereby, the inter-observer reliability was interpreted as 'moderate.' Fleiss' kappa values were also calculated for each rating category, as demonstrated in Table 2.

The students showed poor agreement for atypical cases and fair agreement regarding malignant cases in session A, while benign cases recorded moderate agreement. In session B, all categories showed moderate agreement.

Table 2: Calculated Fleiss' kappa value and the strength of agreement for each category in sessions A and B.

Screen session	Rating category	Fleiss' kappa value	Strength of agreement	Asymptomatic standard error	P-value	95% Confidence Interval (CI)	
						Lower	Upper
A	Benign	0.571	Moderate	0.058	0.000	0.458	0.684
	Atypical	0.166	Poor	0.058	0.004	0.053	0.280
	Malignant	0.289	Fair	0.058	0.001	0.298	0.525
B	Benign	0.553	Moderate	0.058	0.000	0.440	0.666
	Atypical	0.494	Moderate	0.058	0.000	0.381	0.607
	Malignant	0.411	Moderate	0.058	<0.001	0.298	0.525

* κ value <0.20 indicated as 'Poor' agreement, 0.21-0.40 as 'Fair' agreement, 0.41-0.60 as 'Moderate' agreement, 0.61-0.80 as 'Good' agreement and 0.81-1.00 as 'Very good' agreement.

Cohen's kappa was calculated to analyse the intra-observer reliability by comparing the diagnosis data of the two sessions, sessions A and B. Table 3 shows the calculated Cohen's kappa for each student. SO2 had the lowest strength of agreement with a value of 0.395 (fair). In comparison, others show moderate and substantial agreement with values between 0.531 and 0.662. In addition, the results for both Fleiss and Cohen's kappa are statistically significant (P<0.05)

Table 3: The distribution of diagnostic accuracy from each student in sessions A and B.

Students	Cohen's Kappa value	Strength of agreement	p-value
SO1A SO1B	0.652	Substantial	<0.001
SO2A SO2B	0.395	Fair	<0.001
SO3A SO3B	0.544	Moderate	<0.001
SO4A SO4B	0.662	Substantial	<0.001
SO5A SO5B	0.531	Moderate	<0.001
Average value	0.557	Moderate	<0.001

* κ value <0 indicated as 'None' agreement, 0.1-0.20 as 'None to slight' agreement, 0.20-0.41 as 'Fair' agreement, 0.41-0.60 as 'Moderate' agreement, 0.61-0.80 as 'Substantial' agreement and 0.81-1.00 as 'Almost perfect' agreement.

Meanwhile, table 4 shows the diagnostic accuracy of the five students. Based on the table, four students recorded 100% sensitivity and NPV in session A, but it was reduced to only two students in session B.

Table 4: Calculated Cohen's kappa value and the strength of agreement of all students in both screening sessions A and B.

Students	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR	Diagnostic accuracy	p-value
SO1A	100.0	74.1	33.0	100.0	7.288	76.7	0.010
SO1B	100.0	77.8	33.3	100.0	8.048	80.0	0.005
SO2A	66.7	70.4	20.0	95.0	1.556	70	0.197
SO2B	66.7	96.2	50.0	92.6	5.482	90.0	0.004
SO3A	100.0	81.5	37.5	100.0	5.473	90.0	0.002
SO3B	66.7	85.2	33.3	95.8	3.553	83.3	0.033
SO4A	100.0	77.8	33.3	100.0	4.515	80.0	0.005
SO4B	66.7	88.9	40.0	96.0	4.378	86.7	0.014
SO5A	100.0	70.4	27.3	100.0	6.614	70.0	0.016
SO5B	100.0	81.5	37.5	100.0	8.920	83.3	0.002

*PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio

No student records more than 90% in any of the other parameters in both sessions except for SO2. SO2 shows the highest diagnostic accuracy with a value of 90.0% and specificity value (96.2%). However, SO2 exhibits the lowest value for sensitivity (66.7%) and NPV, with a value of 95.0% in session A and 92.6% in session B.

As seen in Table 5, the students performed well, with an average diagnostic accuracy of 80.33%. The average LR and diagnostic accuracy values in the first session were 5.090 and 76.00%. In comparison, the second session shows an average LR value of 6.076 and an average diagnostic accuracy value of 84.66%, which shows an increase in the performance of the students. This result shows a significant value in the diagnostic interpretation of the diagnosis of respiratory cases, although the students were not exposed to hands-on learning beforehand.

Table 5: The average value of the overall performance in both sessions and the total average value for each parameter.

Operating parameters	Screening sessions	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR	Diagnostic accuracy
Average value	A	93.34	74.84	29.62	99.0	5.090	76.00
	B	80.02	85.92	38.82	96.8	6.076	84.66
Total value		86.68	80.38	34.22	97.9	5.583	80.33

*PPV: Positive Predictive Value, NPV: Negative Predictive Value, LR: Likelihood Ratio

Table 6 shows the area AUC value obtained by constructing the ROC curve for all students. The curves that are closer to the left-hand border and the top border of the ROC space indicate higher diagnostic accuracy (N. Roslan et al., 2022). The AUC gave an overall measure of the diagnostic accuracy of the students, which was obtained by plotting the value of sensitivity against 1-specificity. In this ROC curve, the highest value was recorded by SO4 with 0.966, and the lowest value was 0.776, recorded by SO1 in the first session. In the second session, SO4 also had the highest AUC value (0.996) while the lowest was 0.810, which SO1 and SO5 recorded. However, the average AUC values were similar in sessions A and B (0.883).

Table 6: Value of Area under the curve from sessions A and B and their average.

Students	Area under the curve (AUC)		Average
	A	B	
SO1	0.776	0.810	0.793
SO2	0.793	0.931	0.862
SO3	0.931	0.897	0.914
SO4	0.966	0.966	0.966
SO5	0.948	0.810	0.879
Average	0.883	0.883	0.883

The ROC curve for each student is plotted as shown in Figures 2 and 3.

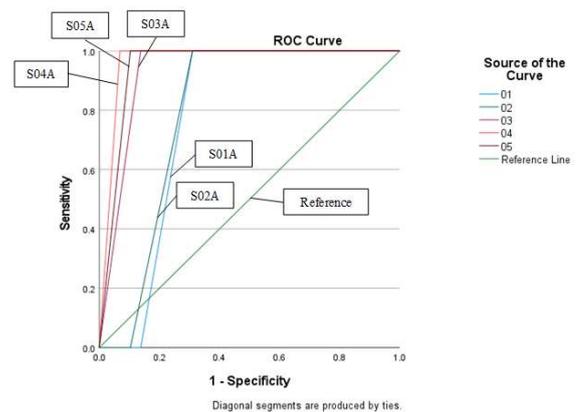


Figure 2: The overall ROC curve of all students in session A.S

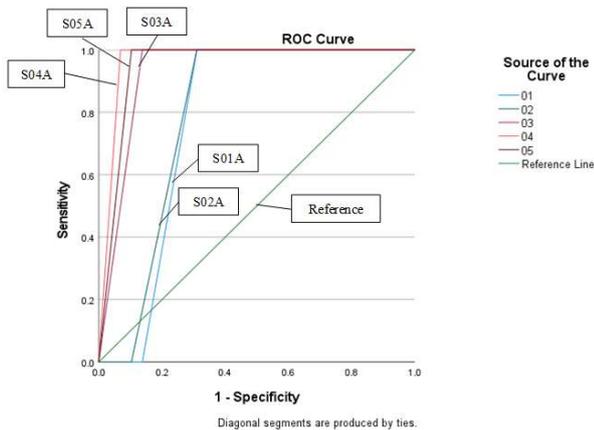


Figure 3: The overall ROC curve of all students in session B.

DISCUSSION

WSI is an emerging technology in digital pathology, though its adoption in cytology remains limited. According to Eccher & Girolami (2020), cytology slides often require Z-stacking capabilities to capture multiple layers of focus for optimal visualization of cell structures. However, this requirement increases both scanning time and data storage demands compared to histology, which makes widespread use of WSI in high-volume cytology workflows challenging and not cost-effective. Thus, WSI has not yet achieved broad implementation for routine diagnostic purposes in cytology.

Nevertheless, WSI provides significant advantages for education and training, particularly with its ability for unlimited, precise annotations on digital slides—a feature not possible with the traditional glass slides. These attributes make WSI a valuable tool in educational settings, where interactive learning experiences and detailed annotations can enhance the training of cytopathologists. As technology advances and storage solutions become more economical, the integration of WSI in cytology may become more feasible, especially in academic and training contexts. In traditional face-to-face practical sessions, students were provided time to observe cytology glass slides through microscopes independently. In contrast, this practical session was conducted online using Google Meet. Since physical observation of glass slides under microscopes was not feasible and the students did not have any access to the microscope, two-way discussions on each case were facilitated between students and the lecturer during the session instead. This setup enabled students to view microscopic specimens in real time while receiving explanations of cell morphology and characteristics from the lecturer. This pedagogical approach is not novel as this was also implemented in another university worldwide. However, despite the availability of virtual microscopy in universities worldwide

for the past few years (Darici et al., 2021), its implementation has yet to be widely adopted by Malaysian universities. Thus, this digital transformation presents a challenge for both educators and students in Malaysia.

Inter-observer reliability

Inter-observer reliability is measured to observe how similar the students score or categorise something, and it is commonly determined using Fleiss' kappa (Nichols et al., 2010). In this research, inter-observer reliability is a method to measure the level of agreement between the students in making a diagnosis. If the consistency between the students is high, it is likely for other similarly trained students to produce similar diagnosis results. The level of agreement used in this study was adapted from Landis & Koch (1977) and Altman (1999), which stated that $\kappa < 0.20$ is interpreted as poor, κ between 0.20 to 0.40 as fair, 0.40 to 0.60 as moderate, 0.60 to 0.80 as good and 0.80 to 1.0 as very good (Mohamad, 2020). Furthermore, Fleiss' kappa for each category was also analysed. Result shows that the students' understanding of atypical and malignant cases was more similar in session B than in session A.

Intra-observer reliability

Intra-observer reliability is a test that assesses the consistency of the students making the diagnosis of the same cases at different times. The screening sessions in this study were conducted at a month gap between them to ensure that the students would not be able to recall the diagnosis they made during the first session. The slides were organised in the same order for both sessions. Based on the results, the students showed 'fair,' 'moderate', and 'substantial' agreement. This shows that the students recognised the cell's characteristics and morphologies and made their diagnoses accordingly (N. Roslan et al., 2022). On the other hand, a similar study on the diagnostic accuracy of respiratory cytology cases reveals contradicting results in which their observers demonstrate 'poor' or 'no' agreement (Malek, 2020). This outcome demonstrates that ODL had no deleterious effect on the students' understanding of respiratory cytology.

Diagnostic accuracy

For diagnostic accuracy measurement, a 2x2 contingency table was constructed. According to previous studies, the results need to be in binary classification for the diagnostic accuracy analysis. For data with non-binary classification, criteria are needed to define the negatives and positives (N. N. Roslan et al., 2021). Thus, the initially benign, atypical, and malignant were divided into two categories:

'benign' and 'malignant'. The benign cases were categorised as 'benign', while atypical and malignant were categorised under 'malignant'.

Overall, the students showed excellent performance, especially in the second session, as they all achieved more than 80.0% diagnostic accuracy. Despite not being able to learn to diagnose respiratory cases under the microscope physically during the ODL study, they were able to diagnose respiratory cases accurately. This result shows that the ODL method was able to give the students enough knowledge and skills to make the proper diagnosis. In addition, this finding is higher than the finding from the previous study, which showed 67.08% diagnostic accuracy (Malek, 2020) and is almost similar to another previous research on thyroid fine-needle aspiration (83.50%) (N. N. Roslan et al., 2021). These findings suggest that the proficiency of students who utilised ODL was comparable to that of individuals who learned similar subjects through traditional methods.

In our research, sensitivity reflects the ability to correctly diagnose positive cases, while specificity reflects the ability to identify negative cases correctly (Trevethan, 2017). The average sensitivity values recorded were 93.34% in the first session and 80.02% in the second session. Moreover, SO1 and SO5 recorded the highest (100%) sensitivity in both sessions. Therefore, SO1 and SO5 showed that they were able to identify all the positive cases correctly. According to the students, malignant cases were easier to identify than benign ones because the appearance of the abnormal cells is easier to identify. Malignant cases are typically distinguishable from benign cases based on the discernible differences in the cells' characteristics. In particular, malignant cells tend to exhibit a high nucleus to cytoplasm ratio (N:C ratio) with two to three times nuclear enlargement compared to benign cells. Additionally, malignant cells often demonstrate hyperchromatic features, irregular coarse chromatin patterns, and dirty backgrounds, among other indicators (Sheaff & Singh, 2013). Generally, the presence of at least three of these malignant characteristics is the minimum criteria for diagnosing a case as malignant. However, it is essential to note that some of these characteristics may present in benign cases due to reactive changes, which can lead to incorrect diagnosis by students.

Although all the results for the operating parameters show a high value, the PPV obtained was low. The results show that the average PPV obtained was 34.22%, with the highest PPV obtained was 50% by SO2 while the lowest was 20%. PPV represents the value of actual positive results among the positive results identified by the students. Since the students did not have enough

experience in diagnosing respiratory cases under a microscope before, they did not have enough confidence to make the diagnoses. Moreover, unlike conventional teaching, the lecturer was not able to provide individual guidance, which is one of the significant contributions to some student's motivation (Mahdzir et al., 2024) and confidence in learning the diagnosis.

The ROC graph generated in this study determines the student's ability to distinguish the positive and negative cases. The AUC values that were obtained from the ROC curve can range from 0 to 1. An AUC value of 1 indicates that the student could perfectly identify positive and negative cases. In contrast, 0 indicates that the student could not identify any cases correctly, which is very unlikely to occur in medical practices. When the AUC value is 0.5 or below, it signifies random chances, which means that the student might be making random guesses while making the diagnosis, which indicates that they do not have the ability to make a correct diagnosis. The average AUC value obtained in this study was 0.883; thus, it can be observed that the students did well differentiating between positive and negative cases. SO4 had the highest AUC value of 0.966, suggesting that SO4 can differentiate positive and negative cases almost perfectly.

Overall, our study revealed that students who participate in the exclusive engagement of ODL have good competency in respiratory cytology slide screening. This statement agrees with another research that underscores that online learning modules have the potential to boost confidence among cytotechnology students, with one student noting that "they do not require much extra training" (Mukherjee & Donnelly, 2018). Another study also highlighted that online learning students can transfer their knowledge to practical skills (Enoch et al., 2022). With more hands-on practical guidance, we believe that the students could make more accurate diagnoses.

LIMITATIONS

One of the limitations of this study is the slides that were obtained from the laboratory. Some of the slides were obtained years ago; thus, there are changes, especially in the appearance of the cell stain. Although the students were briefed about the state of the slides, they might still be confused and assume that the nature of the cases is causal for the observed changes. In addition, since no clinical history was provided for each cytology case, it might affect the student's diagnosis. Moreover, the time required to complete this research was insufficient to ensure that the students did not remember their diagnosis from the first session. Even with a month gap between the sessions, the students might still be likely to remember

some of the cases they screened in the first session. Thus, this may influence the decision they made during the second session. Another limitation is the measuring method used for analysing diagnostic accuracy. Since the measurement of diagnostic accuracy uses the 2x2 contingency table, the only categories that can be used are 'benign' and 'malignant.' The multiple variables, 'benign,' 'atypical', and 'malignant,' could not be used; hence, it does not refer to the actual result.

CONCLUSION

In conclusion, the reliability test shows 'fair' to 'moderate' inter-observer agreement, 'fair' to 'substantial' intra-observer agreement, and an average of 80.33% diagnostic accuracy. Based on these results, it can be concluded that the students can properly diagnose respiratory cases. Even though the students could not experience the hands-on learning of respiratory cytology diagnoses through practical sessions in the lab, they could still diagnose most of the cases correctly. Thus, this study shows that ODL could provide knowledge to the students, and they were competent in screening and diagnosing respiratory cytology cases. However, hands-on learning in the laboratory would increase the students' confidence in making proper respiratory cytology diagnoses.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude to the Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, UiTM Selangor Branch, Malaysia for their unwavering support throughout this research. My heartfelt thanks also goes to the facility and staff for their invaluable assistance and collaboration during the course of this study. It is important to note that this research was not funded by any grant.

REFERENCES

- Darici, D., Reissner, C., Brockhaus, J., & Missler, M. (2021). Implementation of a fully digital histology course in the anatomical teaching curriculum during COVID-19 pandemic. *Annals of Anatomy*, 236, 151-178. <https://doi.org/10.1016/j.aanat.2021.151718>
- Eccher, A., & Girolami, I. (2020). Current state of whole slide imaging use in cytopathology: Pros and pitfalls. *Cytopathology*, 31(5), 372–378. <https://doi.org/10.1111/cyt.12806>
- Enoch, L. C., Abraham, R. M., & Singaram, V. S. (2022). A comparative analysis of the impact of online, blended, and face-to-face learning on medical students' clinical competency in the affective, cognitive, and psychomotor domains. *BMC Medical Education*, 22(1), 1–13. <https://doi.org/10.1186/s12909-022-03777-x>
- Gupta, D. K., Komaromy-Hiller, G., Raab, S. S., & Nath, M. E. (2001). Interobserver and Intraobserver Variability in the Cytologic Diagnosis of Normal and Abnormal Metaplastic Squamous Cells in Pap Smears. *Acta Cytologica*, 45(5), 697–703. <https://doi.org/10.1159/000328290>
- Kumar, N., Gupta, R., & Gupta, S. (2020). Whole Slide Imaging (WSI) in Pathology: Current Perspectives and Future Directions. *Journal of Digital Imaging*, 33(4), 1034–1040. <https://doi.org/10.1007/s10278-020-00351-z>
- Mahdzir, A. A. H. A., Yusof, N. F. M., Helmi, S. A., Pratami, D., & Aisha, A. N. (2024). Impact of Online Learning on Engineering Students' Learning Motivation in Design Classes. *Journal of Advanced Research in Applied Sciences and Engineering Technology*, 37(1), 151-161. <https://doi.org/10.37934/araset.37.1.151161>
- Malek, N. N. A. (2020). Diagnostic Accuracy of Respiratory Cytology and the Reliability of Inter- and Intra-Observer in the Absence of Clinical History.
- Mohamad, S. N. (2020). The Effect of Absence of Clinical History and Demographic Data in Diagnostic Accuracy of Genitourinary Cases Among Slide Observers. *Universiti Teknologi Mara UiTM*.
- Mukherjee, M. S., & Donnelly, A. D. (2018). Initial Assessments of E-Learning Modules in Cytotechnology Education. *Journal of Pathology Informatics*, 9(1),4.https://doi.org/10.4103/jpi.jpi_62_17
- Nichols, T. R., Wisner, P. M., Cripe, G., & Gulabchand, L. (2010). Putting the kappa statistic to use. *Quality Assurance Journal*, 13(3–4), 57–61. <https://doi.org/10.1002/qaj.481>
- Roslan, N., Abu, M., & Ismail, F. (2022). Diagnostic accuracy and agreement between inter- and intra-observers in the absence of clinical history for liquid-based preparation of gynecology specimens. *Journal of Cytology*, 39(3), 110–115. https://doi.org/10.4103/joc.joc_68_22
- Roslan, N. N., Abu, M. N., Abd Malek, N. N., Roslan, N. A., Alihad, N. A., Mohamad, S. N., & Khairil, K. A.

(2021). Effect of absence clinical history in diagnostic accuracy of thyroid fine needle aspiration cytology. *Malaysian Journal of Medicine and Health Sciences*, 17(1), 162–167.

Sheaff, M. T., & Singh, N. (2013). Cytopathology: An introduction. In *Cytopathology: An Introduction*. <https://doi.org/10.1007/978-1-4471-2419-1>

Trevethan, R. (2017). Sensitivity, Specificity, and Predictive Values: Foundations, Liabilities, and Pitfalls in Research and Practice. *Frontiers in Public Health*, 5(November), 1-7. <https://doi.org/10.3389/fpubh.2017.00307>

Zarella, M. D., Bowman, D., Aeffner, F., Farahani, N., Xthona, A., Absar, S. F., Parwani, A., Bui, M., & Hartman, D. J. (2019). A practical guide to whole slide imaging a white paper from the digital pathology association. *Archives of Pathology and Laboratory Medicine*, 143(2), 222–234. <https://doi.org/10.5858/arpa.2018-0343-RA>

Evaluation of Phytochemicals and Antibacterial Activity of *Clinacanthus nutans* (Burm.F.) Lindau Plant

Nurul Izzatul Amiera Rosli¹, Fazleen Haslinda Mohd Hatta², Hartini Yusof^{1*}

¹Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Puncak Alam, Selangor

²Department of Pharmaceutical Pharmacology and Chemistry, Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Puncak Alam, Selangor

ABSTRACT

Background: *Clinacanthus nutans* (Burm.F.) Lindau is a traditionally used medicinal plant, exhibits potential for treating various ailments, including diabetes, cancer, and herpes. However, its antimicrobial properties remain relatively unexplored. This study aimed to investigate the phytochemical constituents and antimicrobial activity of *C. nutans* stem and leaf extracts prepared using ethyl acetate and aqueous solvents. **Methods:** Extracts were evaluated against *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella typhi* through disc diffusion, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC) assays. **Results:** Phytochemical compounds screening revealed the presence of alkaloids, phenols, flavonoids, and saponins in the ethyl acetate extract, while only saponins were detected in the aqueous extract. The ethyl acetate extract demonstrated significant antibacterial activity against all tested bacterial strains, with the largest inhibition zone (14.00 ± 0.00 mm) observed against *S. aureus*. Consistent inhibition was observed across all bacterial strains in the MIC/MBC assays for the ethyl acetate extract. **Conclusion:** The findings suggest that *C. nutans*, particularly the ethyl acetate extract rich in phytochemicals, possesses promising antibacterial properties and warrants further investigation as a potential source for novel antimicrobial agents.

Keywords:

Clinacanthus nutans; antibacterial activity; *Staphylococcus aureus*; *Escherichia coli*; *Salmonella typhi*.

INTRODUCTION

Acanthaceae is one of the most advanced and specialised families, with 250 genera and 2500 species that provide efficient traditional cures for various health issues, and *Clinacanthus nutans* is one of them (Chia et al., 2021). Thus, *C. nutans* which is a herbal medicine or herbs commonly used to deal with illness or liability, has been used as a target study in this research. It is a well-known traditional herb and vegetable that has always been used natively in Southeast Asia, particularly in countries like Malaysia, Thailand, and Indonesia. It has traditionally been used in folk medicine to cure various conditions such as diabetes, cancer, herpes infections, inflammation, and skin disorders (Azemi et al., 2020).

However, despite its traditional use, limited research has explored the specific functions and therapeutic benefits of *C. nutans* leaves in treating various diseases (Shim et al., 2013; Yahaya et al., 2015; Zainol & Mansor, 2019). Given its rich bioactive compounds and historical use in traditional medicine, *C. nutans* presents a compelling subject for scientific investigation, particularly in the search for novel therapeutic agents (Chia et al., 2021). By leveraging the natural compounds within this plant, there is the potential to develop effective and safe antibacterial agents that can combat drug-resistant bacterial infections and improve healthcare outcomes.

The effectiveness and abilities of natural medicine, such as herbs, are indisputable as they have been used for an

* Corresponding author.

E-mail address: tini01@uitm.edu.my

Journal homepage: <https://journals.iium.edu.my/ijahs/index.php/IJAHs>

EISSN NO 2600-8491

exceptionally long time in treating various ailments, even some were used before recorded history (Chuah et al., 2020). Natural resources offer a variety of bioactive substances that may be more effective with less harm than synthesised medications (Mussarat et al., 2021). Recognizing the potential of herbal plants to offer therapeutic benefits with minimal side effects compared to many conventional pharmaceuticals, researchers have shown increasing interest in their investigation. This study focuses on *C. nutans* stems and leaves, driven by a strong desire to expand existing knowledge regarding the plant's phytochemical analysis and antibacterial properties.

In this study, the stems and leaves extract of *C. nutans* was produced using ethyl acetate and distilled water. The extracts were evaluated for their antibacterial activity against *S. aureus*, *E. coli*, and *S. typhi*. In addition, phytochemical analysis was conducted to assess the phytochemical constituents present in the extract.

MATERIALS AND METHODS

Preparation of *C. nutans*

The preparation of *C. nutans* extract began with selecting the healthiest stems and leaves, which were then subjected to the extraction process.

Sample collection

C. nutans plants were collected from Taman Maluri, Kuala Lumpur (3.1342° N, 101.7277° E), Malaysia. Healthy leaves exhibiting upright growth with consistent color and an open surface (not coiled) were selected. Leaves were carefully detached from the stem, ensuring minimal damage. To remove any surface contaminants, each leaf was gently wiped with a dry, sterile tissue. The collected leaves and stems were immediately placed in a clean, zip-lock plastic bag and transported to the laboratory at room temperature for further processing.

Sample processing

C. nutans stems and leaves were cleaned using distilled water to remove all dirt and debris and gently dry them using a soft cloth. The stems and leaves were allowed to dry using an oven for 48 hours at a temperature of 65°C. A mechanical grinder was used to ground the dried stems and leaves into fine powder. The sample was weighed using an analytical scale and stored in Schott bottles and kept in a chiller at a temperature of 4°C.

Extraction of *C. nutans* stems and leaves

C. nutans stems and leaves were powdered and subjected to maceration. A total of 150 grams of powdered plant

material was separately soaked in 600 mL of ethyl acetate and 600 mL of distilled water in conical flasks. The flasks were covered with aluminium foil to minimize solvent evaporation and placed on a platform shaker at 110 rpm for four days at room temperature.

The ethyl acetate extract was filtered through muslin cloth and two consecutive filtrations through 90mm Whatman No. 1 filter paper using cotton gauze. The filtrate was then concentrated to dryness using a rotary evaporator at 40-45°C and 250 mmHg. The concentrated extract was collected in aliquots and dried in a fume hood before being stored at 4°C. As for the aqueous extract, it was filtered twice through muslin cloth and cotton wool. The filtrate was concentrated using a rotary evaporator at 60°C and 150 mmHg. After collecting an initial aliquot, the remaining filtrate was further concentrated and stored overnight at -80°C. The frozen extract was then lyophilised at -53°C. The lyophilized extract was stored at 4°C.

Preparation of *C. nutans* working solution

To prepare the ethyl acetate *C. nutans* working solution, 7.5 grams of the extract was mixed with 7.5 mL of 10% DMSO. Similarly, 11.5 g of the aqueous extract was mixed with 11.5 mL of 10% DMSO. These mixtures resulted in 1000 mg/mL working solutions of *C. nutans* extracts.

Bacterial Culture, Identification, and Confirmation

Bacterial isolation and culture

S. aureus (ATCC: 25923) was cultured on Blood Agar (BA) and Mannitol Salt Agar (MSA). *E. coli* (ATCC: 25922) and *S. typhi* (ATCC: 13311) were cultured on BA and MacConkey agar. Plates were incubated at 37°C for 24 hours, and colony morphology was observed and recorded.

Gram staining

Gram staining was performed on bacterial smears prepared from each isolate. After heat fixation, slides were sequentially stained with crystal violet, Gram's iodine, alcohol, and safranin. Gram-staining characteristics were observed microscopically at 10X and 100X magnification.

Biochemical testing

Catalase and coagulase tests were performed to confirm the identification of *S. aureus*. A series of biochemical tests, including citrate utilization, motility, indole production, methyl red (MR) test, Voges-Proskauer (VP) test, oxidase test, and sugar fermentation tests (TSI, urease), were performed to confirm the identification of *E. coli* and *S. typhi*.

Antimicrobial Susceptibility Testing

Inoculum preparation

A few colonies of tested organisms were inoculated into 5 mL of Muller-Hinton broth (MHB). Later, the broth was incubated at 37°C for 24 hours. After the incubation period, the turbidity of the broth was compared with the 0.5 suspension of McFarland standard.

Well diffusion testing

Sterile cotton swabs were used to inoculate MHB with the respective bacterial strains. The inoculated MHB was then used to lawn Muller-Hinton agar (MHA) plates. Three wells (0.6 mm diameter) were bored into each inoculated agar plate using a sterile cork borer. Subsequently, 50 µL of *C. nutans* stem and leaf extracts were added to one well, and 50 µL of 10% DMSO served as the negative control in another well. A standard antibiotic disc (Tetracycline (TE30) for *S. aureus*, Gentamicin (CN10) for *E. coli*, and Ampicillin (AMP10) for *S. typhi*) was placed in the third well as a positive control. The inoculated plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones around each well was measured in millimetres using a transparent ruler, and the results were recorded. The average inhibition zone diameter for each extract was calculated.

Minimum Inhibitory Concentration

Inoculum preparation

A few colonies of each organism were introduced into MHB. The medium was placed into the incubator to allow bacteria to grow at 37°C for 24 hours. The suspension's turbidity was adjusted to 0.5 McFarland after incubation.

Broth microdilution method

MIC testing was performed within a biosafety cabinet. Two-fold serial dilutions of *C. nutans* stem and leaf extracts were prepared, starting with the highest concentration of 1000 mg/mL. Fifty µL of MHB was dispensed into wells 1-12 of a 96-well microtiter plate. Subsequently, 50 µL of diluted plant extract was added to wells 1-10, resulting in a gradient of decreasing concentrations. A total of 50 µL of microbial suspension (5×10^5 CFU/mL) was then added to wells 1-10. Wells 11 and 12 served as controls: well 11 contained the selected bacterial strain (positive control), and well 12 contained 10% DMSO (negative control). The microtiter plates were mixed thoroughly and incubated at 37°C. Microbial growth was assessed visually by observing turbidity and pellet formation at the bottom of the wells. The MIC endpoint was determined as the lowest concentration of plant extract that completely inhibited visible bacterial growth, which was identified as the well preceding the first well showing turbidity. Each test was

performed in triplicate to obtain the mean MIC values for each bacterial strain.

Minimal Bactericidal Concentration

The aliquot of 10 µL from all tubes with no visible growth was proceeded with the minimum bactericidal concentration (MBC). The bacteria were suspended in MHB medium and cultured on MHA medium. Later, the medium was incubated at 37°C for 24 hours. The growth of bacteria was observed and recorded. The MBC endpoint values were determined based on the lowest concentration of *C. nutans* extract, which inhibits 99.9% of the initial bacterial growth.

Interpretation of Data

The AST result was analysed using One-Way ANOVA. Statistical Package for the Social Sciences (SPSS) programme was used to analyse the data.

RESULTS

***C. nutans* (Burm.F.) Lindau Crude Extract**

The ethyl acetate extract from *C. nutans* exhibited a dark green, viscous appearance with a glossy sheen (Figure 1(a)). Initially, the extract displayed a dark colouration with a thin, watery consistency, gradually thickening and becoming more viscous. A yield of 7.5g of crude extract was obtained from 150g of plant powder.



Figure 1 (a): Ethyl acetate crude extract of *C. nutans* (Burm.F.) Lindau appearance.

The aqueous extract from *C. nutans* exhibited a mahogany brown appearance (Figure 1(b)). Initially, the extract displayed a lighter brown colouration with a thin, watery consistency. However, it gradually thickened and became more viscous. A yield of 11.5g of crude extract was obtained from 150g of plant powder.



Figure 1 (b): Aqueous solution (distilled water) crude extract of *C. nutans* (Burm.F.) Lindau appearance

The results from Table 1 and Figure 2 collectively indicate that both ethyl acetate and aqueous extracts of *C. nutans* possess significant antibacterial activity, with the ethyl acetate extract demonstrating superior inhibitory effects against *S. aureus*, *E. coli*, and *S. typhi* compared to the aqueous extract. While standard antibiotics exhibited generally higher potency, statistical analysis confirmed significant differences between the plant extracts and controls, validating the observed antibacterial properties of the *C. nutans* extracts.

Antimicrobial Susceptibility Testing

Table 1: Mean diameter zone of inhibition for selected bacteria

Bacteria	Ethyl acetate extract			Aqueous extract			Tetracycline ^a (10 µg)	Gentamicin ^a (30 µg)	Ampicillin ^a (30 µg)	DMSO ^b
	Mean diameter (SD)	F-statistic ^a (df)	P-value	Mean diameter (SD)	F-statistic ^a (df)	P-value				
<i>S. aureus</i> (ATCC: 25923)	14.00 (0.00)	3913.00 (2)	<0.001 ^b	6.00 (0.00)	3913.00 (2)	<0.001 ^b	29.67±0.57	-	--	6.00*±0.00
<i>E. coli</i> (ATCC: 25922)	12.33 (0.57)	327.88 (2)	<0.001 ^b	6.00 (0.00)	327.88 (2)	<0.001 ^b	-	25.33±1.53	--	6.00*±0.00
<i>S. typhi</i> (ATCC: 13311)	12.67 (2.52)	127.27 (2)	<0.001 ^b	6.00 (0.00)	127.27 (2)	<0.001 ^b	-	-	22.00±1.00	6.00*±0.00

(^a) positive control, (^b) negative control, (*) diameter of well, (SD) standard deviation, (-) not tested.

^a One-Way ANOVA test

^b Only 'Ethyl acetate and Aqueous extract against standards antibiotic' of all selected bacteria (<0.001) pairs are significantly different by post-hoc test Scheffe's procedure.

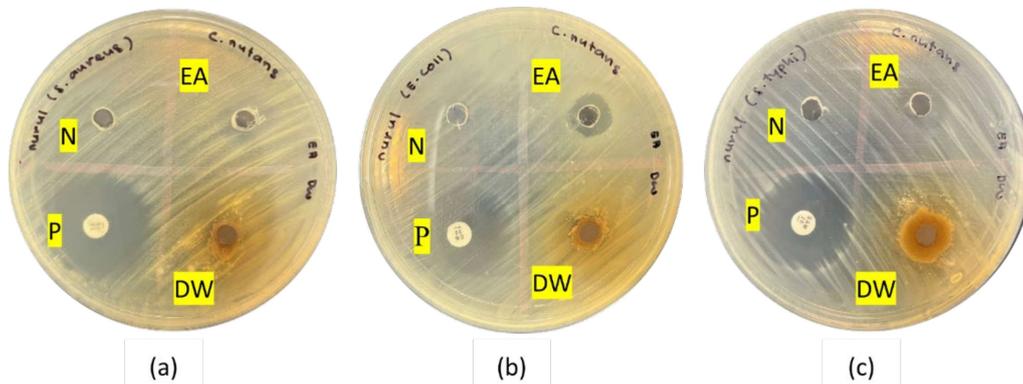


Figure 2: Growth inhibition of tested bacteria (a) test on *S. aureus*, (b) test on *E. coli*, (c) test on *S. typhi*, (EA) ethyl acetate plant extract, (DW) distilled water plant extract, (P) positive control, and (N) negative control.

Minimum Inhibitory Concentration (MIC) And Minimal Bactericidal Concentration (MBC)

Table 2 presents the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values for ethyl acetate extracts of *C. nutans* stems and leaves against *S. aureus*, *E. coli*, and *S. typhi*. Across all tested bacterial strains, the MIC was consistently

determined to be 500 mg/mL, while the MBC was 1000 mg/mL. This consistent finding results in an MBC/MIC ratio of 2 for all three bacterial species, suggesting a bactericidal effect of the plant extract, as an MBC/MIC ratio of ≤ 4 is generally indicative of bactericidal activity.

Table 2: Mean values for MIC and MBC of ethyl acetate extracts of *C. nutans* stems and leaves

Bacteria	Concentration of plant extract (mg/mL)		MBC/MIC ratio
	MIC	MBC	
<i>S. aureus</i> (ATCC: 25923)	500	1000	2
<i>E. coli</i> (ATCC: 25922)	500	1000	2
<i>S. typhi</i> (ATCC: 13311)	500	1000	2

Qualitative Phytochemical Analysis

Table 3: Phytochemicals screening of *C. nutans* stems and leaves extracts

Bioactive Compounds	Ethyl acetate extract	Aqueous extract
Alkaloids	Present	Absent
Phenols	Present	Absent
Flavonoids	Present	Absent
Saponins	Present	Present
Glycosides	Absent	Absent
Steroids	Absent	Absent

Phytochemicals screening revealed the presence of alkaloids, phenols, flavonoids, and saponins in the ethyl acetate extract, while all these compounds were absent in the aqueous extract except saponins.

DISCUSSION

Figures 1 (a) and (b) illustrate the distinct physical characteristics of the crude ethyl acetate and aqueous extracts of *C. nutans*, providing initial insights into the nature of the compounds extracted by solvents of differing polarities. Both extracts initially displayed a thin, watery consistency that gradually thickened and became more viscous upon standing. This change in viscosity for both extracts could be attributed to the evaporation of the respective solvents, leading to a higher concentration of the extracted plant material.

The ethyl acetate extract, depicted in Figure 1 (a), presented as a dark green, viscous substance with a glossy sheen. This appearance suggests the successful extraction of less polar compounds, which are often chlorophylls; contributing to the green color and various lipophilic

secondary metabolites which resulted in viscous, resinous consistency. The percentage of yield was 5% as from 150g of plant powder, a yield of 7.5g of this extract was obtained.

In contrast, Figure 1 (b) showcased the aqueous extract as mahogany brown. The aqueous extract yielded a higher amount, 11.5g from the same quantity of plant powder (7.7%), indicating that water, being a highly polar solvent, extracted a greater mass of water-soluble compounds. These visual and yield differences are crucial as they prefigure the distinct phytochemical profiles and varying biological activities observed in subsequent analyses, directly reflecting the selectivity of the extraction solvents for different classes of plant secondary metabolites (Noorazlina et al., 2024).

S. aureus (ATCC 25923), *E. coli* (ATCC 25922), and *S. typhi* (ATCC 13311) were the selected bacteria species tested for the antimicrobial activity of both ethyl acetate and aqueous extract of *C. nutans* stems and leaves. Utilizing a pure bacterial strain is essential for ensuring the accuracy and precision of antimicrobial susceptibility testing. The ethyl acetate extract of *C. nutans* demonstrated significant antibacterial activity against all tested bacterial strains, with *S. aureus* exhibiting the largest inhibition zone (14.00 ± 0.00 mm). However, the observed inhibition zones were smaller compared to the positive control antibiotics (Tetracycline, Gentamicin, and Ampicillin), as indicated by the statistically significant difference ($p < 0.001$) between the plant extracts and the positive controls.

To further evaluate the antimicrobial potency, MIC and MBC determinations were performed. The ethyl acetate extract exhibited a MIC of 500 mg/mL against all tested strains, demonstrating consistent antibacterial activity. Notably, all strains' MBC/MIC ratios were less than 4, indicating bactericidal activity. In contrast, the high-water content of the aqueous extract hindered accurate MIC determination using the current methodology. Future studies should employ alternative methods, such as microdilution techniques, to more precisely determine the MIC and MBC values for the aqueous extract (Thuille et al., 2003; Xiang & Xian, 2023).

Through this study, the ethyl acetate extract of *C. nutans* demonstrated superior antibacterial activity compared to the aqueous extract. The observed superior antibacterial activity of the ethyl acetate extract, particularly against *S. aureus* (14.00 mm inhibition zone), aligns with previous findings demonstrating superior antibacterial efficacy of non-polar extracts of *C. nutans* against gram-positive bacteria (Silva et al., 2009; Lim et al., 2020). While the ethyl

acetate extract demonstrated promising antibacterial activity, its potency appeared lower than that of conventional antibiotics. This could be attributed to several factors, including compound concentration and different mechanisms of action between plant extracts and synthetic antibiotics.

The observed bactericidal activity of the ethyl acetate extract can be attributed to the presence of multiple phytochemical constituents with antimicrobial properties. Antibacterial substances can be classified into six main classes according to their modes of action. These methods encompass the prevention of cell wall formation, alterations to the structural integrity of the plasma membrane, interference with cellular energy production, impairment of nucleic acid synthesis, disruption of protein synthesis, and adjustments to crucial metabolic pathways (Singh et al., 2021; Eshboev et al., 2024). However, the precise mechanisms underlying the antimicrobial activity of *C. nutans* extracts require further investigation.

It was noted that despite the higher yield obtained with aqueous extraction, the ethyl acetate extract exhibited superior antimicrobial activity, indicating that the semi-polar nature of ethyl acetate facilitates the extraction of the bioactive compounds responsible for these antimicrobial effects. Ethyl acetate is also believed to extract the highest active compounds due to polarity-differentiated solvents, besides dichloromethane and water (Dang et al., 2024). These findings align with previous research indicating that different solvent polarities can selectively extract various classes of phytochemicals (Dirar et al., 2019) and the phytochemical profile of plant extracts is significantly influenced by the extraction method and solvent polarity. As demonstrated by Sabindo et al. (2024), utilizing different solvents, such as water, ethanol, and methanol, can yield diverse phytochemical compositions, including additional classes like steroids, triterpenoids, glycosides, and phytosterols. This highlights the importance of solvent selection in optimizing the extraction of specific bioactive compounds from plant matrices.

The *C. nutans* plant has been subjected to numerous phytochemical analyses, revealing the presence of a diverse array of bioactive chemicals (Alam et al., 2016). These compound classes are known for their diverse pharmacological activities, including antimicrobial, antioxidant, and anti-inflammatory properties (Shaikh & Patil, 2020). The findings of this study were not consistent with the previous study, where flavonoids, triterpenoids, steroids, phytosterols, and glycosides are the phytochemical classes most likely present in *C. nutans* extract when the plant is extracted using a polar solvent

such as water, methanol, ethanol, or aqueous organic solvents, or by a semipolar solvent such as chloroform. Alkaloids, saponins, and tannins in *C. nutans* depend on the plant's origin and the post-harvesting method. For instance, alkaloids were typically present in water and chloroform leaf extracts but were absent in methanol and aqueous methanol leaf extracts collected from Malaysia (Khoo et al., 2018). These findings contradict a previous study suggesting alkaloids and flavonoids in aqueous extracts of *C. nutans* (Xiang & Xian, 2023). Besides, younger *C. nutans* leaves exhibit higher phenolic levels than mature leaves, emphasizing the importance of plant age in phytochemical profiling (Chia et al., 2021). The absence of these compounds in the aqueous extract further emphasizes the importance of solvent selection in optimizing phytochemical extraction.

The presence of alkaloids, phenols, flavonoids, and saponins in the ethyl acetate extract correlates with its enhanced antimicrobial activity. Alkaloids hinder the growth of bacteria by employing various mechanisms, such as obstructing the synthesis of bacterial nucleic acid and proteins, altering the permeability of the bacterial cell membrane, causing damage to the cell membrane and cell wall, impeding bacterial metabolism, and obstructing efflux pumps (Zhang et al., 2020; Yan et al., 2021). Nucleic acids, which contain the genetic material required for reproduction and protein synthesis, are essential components of bacteria. Alkaloids have a low-to-moderate molecular weight (250–600 Daltons); they are water-soluble and lipid soluble under either acidic or neutral and basic conditions, respectively. Research on alkaloid cell membrane interaction explained the possible mechanism of alkaloids penetration through the cell membrane. The results suggested that the alkaloid–lipid interaction affects the lipid headgroup structure by weakening the lipid headgroup–headgroup H bonds and enhancing the electrostatic attraction between the alkaloids and the lipid phosphate groups, facilitating the alkaloid passage between the headgroups (Abookleesh et al., 2022). Hence, natural alkaloids can effectively combat various bacteria, including Methicillin-Resistant *Staphylococcus aureus* (MRSA), a commonly found pathogen responsible for clinical illnesses. These alkaloids are currently used as primary chemicals to advance novel antibacterial medications (Yan et al., 2021).

Interestingly, alkaloids have also been shown to potentially inhibit bacterial efflux pumps. Mohtar et al. (2009) demonstrated the efficacy of 13 antibacterial alkaloids as efflux pump inhibitors (EPIs) against a panel of three MRSA strains. Antibiotic efflux pumps are membrane proteins that actively transport antibiotics and other harmful compounds from bacterial cells, contributing

significantly to antibiotic resistance. These pumps play crucial roles in bacterial physiology, including removing antibiotics, quorum-sensing molecules, and virulence factors (Gaurav et al., 2023). Bacteria can reduce intracellular drug concentrations by effluxing antibiotics, thus hindering antibiotic efficacy and promoting resistance (Yan et al., 2021).

Previous studies have demonstrated that phytochemicals such as phenols, flavonoids, and saponins possess antibacterial properties, primarily by disrupting bacterial cell membranes (Cueva et al., 2010; Khan et al., 2018; Kauffmann & Castro, 2023). The bacterial cell membrane, composed of phospholipids and proteins, lacks sterols due to the absence of mitochondria in prokaryotes. This membrane is critical in various cellular functions, including nutrient transport, waste removal, and maintaining cellular homeostasis. The interaction between phytochemicals and the bacterial cell membrane can lead to significant alterations in membrane permeability. This disruption can compromise cellular integrity, leading to the leakage of essential intracellular components and cell death. Furthermore, damage to the bacterial cell wall, a rigid structure that provides structural support and maintains cell shape, can also contribute to bacterial demise. Weakening the peptidoglycan layer, a key component of the bacterial cell wall can lead to osmotic imbalance and subsequent cell lysis. This phenomenon, often triggered by antibiotics, phage infection, or activation of bacterial endolysins, releases cellular contents, including proteins, DNA, and membrane vesicles, ultimately leading to bacterial death (Villageliu & Samuelson, 2022). These biologically active compounds demonstrate potential in overcoming the development of resistance in bacterial pathogens and fighting against bacterial illnesses. These chemicals could enhance the effectiveness of traditional antibiotics and prevent the emergence of resistance, thereby allowing them to be used in medical treatments again (Khameneh et al., 2021; Kumar et al., 2023).

CONCLUSION

This study provides evidence for the antibacterial potential of *C. nutans* (Burm.F.), particularly the ethyl acetate extract derived from its stems and leaves. The observed inhibition of *S. aureus*, *E. coli*, and *S. typhi* supports the plant's traditional use in treating bacterial infections. While the ethyl acetate extract showed greater efficacy against gram-positive bacteria, further investigations are warranted.

ACKNOWLEDGEMENT

The authors would like to acknowledge Faculty of Health Sciences, Universiti Teknologi MARA (UiTM) for supporting

this research.

REFERENCES

- Abookleesh, F. L., Al-Anzi, B. S., & Ullah, A. (2022). Potential antiviral action of alkaloids. *Molecules/Molecules Online/Molecules Annual*, 27(3), 903. <https://doi.org/10.3390/molecules27030903>
- Alam, A., Ferdosh, S., Ghafoor, K., Hakim, A., Juraimi, A. S., Khatib, A., & Sarker, Z. I. (2016). *Clinacanthus Nutans*: A review of the medicinal uses, pharmacology and phytochemistry. *Asian Pacific Journal of Tropical Medicine*, 9(4), 402–409. <https://doi.org/10.1016/j.apjtm.2016.03.011>
- Azemi, A. K., Mokhtar, S. S., & Rasool, A. H. (2020). *Clinacanthus nutans*: Its potential against diabetic vascular diseases. *Brazilian Journal of Pharmaceutical Sciences*, 56. <https://doi.org/10.1590/s2175-97902020000118838>
- Chia, T. Y., Gan, C. Y., Murugaiyah, V., Hashmi, S. F., Fatima, T., Ibrahim, L., Abdulla, M. H., Alswailmi, F. K., Johns, E. J., & Ahmad, A. (2021). A narrative review on the phytochemistry, pharmacology and therapeutic potentials of *Clinacanthus Nutans* (Burm. f.) lindau leaves as an alternative source of future medicine. *Molecules*, 27(1), 139. <https://doi.org/10.3390/molecules27010139>
- Chiu, H. I., Mood, C. N. a. C., Zain, N. N. M., Ramachandran, M. R., Yahaya, N., Kamal, N. N. S. N. M., Tung, W. H., Yong, Y. K., Lee, C. K., & Lim, V. (2021). Biogenic Silver Nanoparticles of *Clinacanthus nutans* as Antioxidant with Antimicrobial and Cytotoxic Effects. *Bioinorganic Chemistry and Applications/Bioinorganic Chemistry and Applications*, 2021, 1–11. <https://doi.org/10.1155/2021/9920890>
- Chuah, P. N., Nyanasegaram, D., Yu, K.-X., Mohamed Razik, R., Al-Dhalli, S., Kue, C. S., Shaari, K., & Ng, C. H. (2020). Comparative conventional extraction methods of ethanolic extracts of *clinacanthus nutans* leaves on antioxidant activity and toxicity. *British Food Journal*, 122(10), 3139–3149. <https://doi.org/10.1108/bfj-02-2020-0085>
- Cueva, C., Moreno-Arribas, M. V., Martín-Álvarez, P. J., Bills, G., Vicente, M. F., Basilio, A., Rivas, C. L., Requena, T., Rodríguez, J. M., & Bartolomé, B. (2010). Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria.

- Research in Microbiology, 161(5), 372–382. <https://doi.org/10.1016/j.resmic.2010.04.006>
- Dang, K. D., Nguyen, C. H. M., Nguyen, T. N. N., & Nguyen, D. T. T. (2024). Antioxidant potentials of extracts from different parts of *Clinacanthus nutans* (Burm. f.) Lindau. *Pakistan journal of pharmaceutical sciences*, 37(2(Special)), 451–458
- Dirar, A., Alsaadi, D., Wada, M., Mohamed, M., Watanabe, T., & Devkota, H. (2019). Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants. *South African Journal of Botany*, 120, 261–267. <https://doi.org/10.1016/j.sajb.2018.07.003>
- Eshboev, F., Mamadalieva, N., Nazarov, P. A., Hussain, H., Katanaev, V., Egamberdieva, D., & Azimova, S. (2024). Antimicrobial Action Mechanisms of Natural Compounds Isolated from Endophytic Microorganisms. *Antibiotics*, 13(3), 271. <https://doi.org/10.3390/antibiotics13030271>
- Gaurav, A., Bakht, P., Saini, M., Pandey, S., & Pathania, R. (2023). Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology*, 169(5). <https://doi.org/10.1099/mic.0.001333>
- Kauffmann, A. C., & Castro, V. S. (2023). Phenolic Compounds in Bacterial Inactivation: A Perspective from Brazil. *Antibiotics*, 12(4), 645. <https://doi.org/10.3390/antibiotics12040645>
- Khameneh, B., Eskin, N. a. M., Iranshahy, M., & Bazzaz, B. S. F. (2021). Phytochemicals: A Promising Weapon in the Arsenal against Antibiotic-Resistant Bacteria. *Antibiotics*, 10(9), 1044. <https://doi.org/10.3390/antibiotics10091044>
- Khan, M. I., Ahmed, A., Shin, J. H., Baek, J. S., Kim, M. Y., & Kim, J. D. (2018). Green Tea Seed Isolated Saponins Exerts Antibacterial Effects against Various Strains of Gram Positive and Gram Negative Bacteria, a Comprehensive Study In Vitro and In Vivo. *Evidence-based Complementary and Alternative Medicine*, 2018, 1–12. <https://doi.org/10.1155/2018/3486106>
- Khoo, L. W., Audrey Kow, S., Lee, M. T., Tan, C. P., Shaari, K., Tham, C. L., & Abas, F. (2018). A comprehensive review on phytochemistry and pharmacological activities of *clinacanthus nutans* (burm.f.) Lindau. *Evidence-Based Complementary and Alternative Medicine*, 2018, 1–39. <https://doi.org/10.1155/2018/9276260>
- Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M., Elobeid, T., K, S., & Oz, F. (2023). Major Phytochemicals: recent advances in health benefits and extraction method. *Molecules/Molecules Online/Molecules Annual*, 28(2), 887. <https://doi.org/10.3390/molecules28020887>
- Lim, S. E., Almakmari, M. A., Alameri, S. I., Chin, S., Abushelaibi, A., Mai, C., & Lai, K. (2020). Antibacterial Activity of *Clinacanthus nutans* Polar and Non-Polar Leaves and Stem Extracts. *Biomedical & Pharmacology Journal*, 13(03), 1169–1174. <https://doi.org/10.13005/bpj/1984>
- Mohtar, M., Johari, S. A., Li, A. R., Isa, M. M., Mustafa, S., Ali, A. M., & Basri, D. F. (2009). Inhibitory and Resistance-Modifying Potential of Plant-Based Alkaloids Against Methicillin-Resistant *Staphylococcus aureus* (MRSA). *Current Microbiology*, 59(2), 181–186. <https://doi.org/10.1007/s00284-009-9416-9>
- Mussarat, S., Ali, R., Ali, S., Mothana, R. A., Ullah, R., & Adnan, M. (2021). Medicinal animals and plants as alternative and complementary medicine in southern regions of Khyber Pakhtunkhwa, Pakistan. *Frontiers in Pharmacology*, 12. <https://doi.org/10.3389/fphar.2021.649046>
- Noorazlina, A., Muhammad Sulaiman, M. J., Raihana, M. Y., Nurul Iman, A., Nurul Nadiah, H., & Aisyah Salihah, K. (2024). Comparative Study on Different Extraction Methods for Stilbenoids: Maceration and Supercritical Fluid Extraction (SFE) of *Anisoptera laevis* Ridl. *MALAYSIAN JOURNAL of CHEMISTRY*, 26(1). <https://doi.org/10.55373/mjchem.v26i1.1>
- Sabindo, N. H., Yatim, R. M., & Ponnuraj, K. T. (2024). Phytochemical composition of *Clinacanthus nutans* based on factors of environment, genetics, and postharvest processes: A review. *Biomedicine*, 14(2). <https://doi.org/10.37796/2211-8039.1451>
- Shaikh, J. R., & Patil, M. (2020). Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*, 8(2), 603–608. <https://doi.org/10.22271/chemi.2020.v8.i2i.8834>
- Shim, S.Y., Aziana, I. & Khoo, B.Y. (2013). Perspective and insight on *Clinacanthus nutans* Lindau in traditional

medicine. *International Journal of Integrative Biology* 14(1): 7-9.

Silva, I. E., Junior, Filho, V. C., Zacchino, S. A., Da S Lima, J. C., & De O Martins, D. T. (2009). Antimicrobial screening of some medicinal plants from Mato Grosso Cerrado. *Revista Brasileira De Farmacognosia*, 19(1b), 242–248. <https://doi.org/10.1590/s0102-695x2009000200011>

Singh, S. P., Qureshi, A., & Hassan, W. (2021). Mechanisms of action by antimicrobial agents: A review. *McGill Journal of Medicine*, 19(1). <https://doi.org/10.26443/mjm.v19i1.217>

Thuille, N., Fille, M., & Nagl, M. (2003). Bactericidal activity of herbal extracts. *International Journal of Hygiene and Environmental Health*, 206(3), 217–221. <https://doi.org/10.1078/1438-4639-00217>

Villageliu, D. N., & Samuelson, D. R. (2022). The role of bacterial membrane vesicles in human health and disease. *Frontiers in Microbiology*, 13. <https://doi.org/10.3389/fmicb.2022.828704>

Xiang, T. K. & Xian, C. S. (2023). Phytochemical Screening and Antibacterial Properties of Methanol, Hexane and Water Extracts of *Clinacanthus nutans*. *Malaysian Journal of Chemistry*, 2023, Vol. 25(4), 199–206. <https://ikm.org.my/publications/malaysian-journal-of-chemistry/xcesfile.php?abs=J0045-830d5cf>

Yahaya, R., Dash, G.K., Abdullah, M.S. & Mathews, A. (2015). *Clinacanthus nutans* (burm. F.) Lindau: An useful medicinal plant of south-east Asia. *Int. J. Pharmacogn. Phytochem.* 7, 1244–1250

Yan, Y., Li, X., Zhang, C., Lv, L., Gao, B., & Li, M. (2021). Research Progress on Antibacterial Activities and Mechanisms of Natural alkaloids: a review. *Antibiotics*, 10(3), 318. <https://doi.org/10.3390/antibiotics10030318>

Zainol, H., & Mansor, H. (2019). A review of therapeutic potentials of *Clinacanthus Nutansas* source for alternative medicines. *Sains Malaysiana*, 48(12), 2683–2691. <https://doi.org/10.17576/jsm-2019-4812-09>

Zhang, Q., Lyu, Y., Huang, J., Zhang, X., Yu, N., Wen, Z., & Chen, S. (2020). Antibacterial activity and mechanism of sanguinarine against *Providencia rettgeri* in vitro. *PeerJ*, 8, e9543. <https://doi.org/10.7717/peerj.9543>

Knowledge and Awareness of Hand, Foot, and Mouth Disease (HFMD) Among Parents in Kampung Tuan Mandak, Terengganu

Rabiatul Adawiah Mohamad Asri and Mazura Bahari*

^{1,1}Center for Medical Laboratory Technology Studies, Faculty of Health Sciences, UiTM Cawangan Selangor (UCS), 42300 Puncak Alam Campus, Selangor, MALAYSIA

²Research and Education in Biomedical, Epidemiology and Immunology Aliances (REBEIA), FSK, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Background: Hand, foot, and mouth disease (HFMD) is endemic in Malaysia, primarily affecting children under five. Recent surges in HFMD cases have exceeded the national alert threshold of 1,150 cases per week. This study aimed to assess the level of knowledge and awareness of HFMD among parents in Kampung Tuan Mandak, Terengganu. **Methods:** A cross-sectional study was conducted from April to June 2023 using a self-administered online questionnaire distributed via QR codes and social media platforms. A total of 279 parents participated through simple random sampling. Descriptive statistics were used to analyze socio-demographic data, knowledge, and awareness levels, while one-way ANOVA assessed differences across educational backgrounds. **Results:** Findings revealed that 61.3% of parents had poor knowledge of HFMD, and 42.7% demonstrated low awareness. Only 9% had good knowledge, while 35.8% showed good awareness. Interestingly, educational attainment did not significantly correlate with higher knowledge or awareness levels. **Conclusion:** These results underscore the importance of tailored health education initiatives focused on HFMD transmission, symptoms, and prevention. Enhancing public health communication, particularly in rural communities, may help strengthen parental understanding and support ongoing disease prevention efforts.

Keywords:

awareness, HFMD, knowledge, parents

INTRODUCTION

Hand, foot, and mouth disease (HFMD) is a common viral illness that primarily affects infants and young children. It is caused by viruses from the *Enterovirus* genus of the *Picornaviridae* family, including Coxsackieviruses A and B, Echoviruses, Polioviruses, and Enteroviruses (Charoenchokpanit & Pumpaibool, 2013; Mansur & Ahmad, 2021). Although HFMD is usually mild and not life-threatening, it is highly contagious (Rajamoorthy et al., 2022). Most infections are asymptomatic or present with mild symptoms such as fever, rashes, blisters, lethargy, upper respiratory issues, and lesions (Chang et al., 2011).

HFMD typically begins with a mild fever, followed by papulovesicular rashes, herpangina, and sometimes onychomadesis. Rashes commonly appear on the hands, feet, knees, and buttocks. Fever usually lasts 3–4 days, mouth sores about 7 days, and skin lesions up to 10 days. Diagnosis is confirmed through stool or blood samples (Mahadzar & Rahman, 2019). HFMD is a global public

health concern, particularly in the Asia-Pacific region, affecting mainly children under five. China reports over 2 million cases annually. The most common causative agents are Coxsackievirus A16 and EV71 (Puenpa et al., 2019). In settings like daycares, the disease spreads rapidly via contact with respiratory secretions, blister fluid, or feces. Developing countries face more challenges due to limited healthcare access. In China, EV71 vaccines and public health campaigns have shown promise in reducing transmission and severe cases (Li et al., 2019). Since it was first identified in New Zealand in 1957, HFMD has caused several major outbreaks globally. Notable incidents include the 1998 Taiwan outbreak and subsequent outbreaks in China, Vietnam, and Malaysia, which emphasized the need for improved prevention and control strategies (Koh et al., 2016). Malaysia's first significant outbreak occurred in 1997 in Sarawak, affecting over 2,600 children and causing several deaths due to EV71 (World Health Organization, 1997). In 2018, Malaysia's Ministry of Health reported 29,358 HFMD cases, a significant rise from the previous year (Ismail et al., 2021).

* Corresponding author.

E-mail address: mazurabahari@uitm.edu.my

By mid-May 2022, over 31,661 cases had been reported in Malaysia—a 15-fold increase compared to the same period in 2021. This surge highlighted HFMD as a growing public health threat, driven by better awareness, enhanced surveillance, and the disease’s epidemiological trends (Ministry of Health Malaysia, 2022a). Studies show a notable gap in HFMD awareness between urban and rural communities. Urban areas tend to have better access to healthcare and public education campaigns, while rural areas like Kampung Tuan Mandak face challenges such as limited services, low health literacy, and reduced outreach (Noor & Remo, 2020). In Malaysia, urban states like Selangor and Kuala Lumpur report higher HFMD cases due to population density and childcare attendance. In contrast, rural Terengganu reports fewer, more localized cases (Ministry of Health Malaysia, 2022b).

This study investigates HFMD knowledge and awareness among parents in Kampung Tuan Mandak, Terengganu, a rural Malaysian community where limited healthcare access, inadequate public health outreach, and cultural practices may hinder timely treatment and awareness (World Health Organization, 2021). Despite the growing significance of HFMD, no prior research has examined awareness levels in this area. This study addresses that gap by assessing parental knowledge and comparing it across educational backgrounds, offering insights to guide targeted health education strategies.

MATERIALS AND METHODS

A cross-sectional survey was conducted from April to June 2023, following approval from the Research Ethics Committee, Universiti Teknologi MARA, Selangor (Ref: FERC/FSK/MR/2023/00071). An online questionnaire was administered via Google Forms, with survey links and QR codes distributed through social media platforms including WhatsApp, Telegram, Instagram, and Facebook. The study targeted residents of Kampung Tuan Mandak, a rural Malaysian community selected for its limited access to healthcare and health education compared to urban areas. The study aimed to assess awareness and preventive practices related to HFMD in such underserved populations. Participation was voluntary, with informed consent obtained from all respondents. Eligible participants were male and female residents aged 18–59 with varying education and income levels. Individuals who were technologically illiterate or declined participation were excluded.

Sample Design and Setting

Sample selection

Residents of Kampung Tuan Mandak aged 18 to 59 were randomly selected to participate in the study, including both males and females with diverse educational backgrounds and household incomes. Individuals who were technologically illiterate or unwilling to participate were excluded.

Sample size

The sample size of this study was determined by the following statistics formula:

$$n = \frac{N}{1 + t N(e)^2}$$

Where,

n= sample size

N= population size

e= level of precision

Hence,

$$= \frac{675}{1 + 675 (0.05)^2}$$

Sample size= 251 respondents

If 10% non-response rate, = 278.88

The study requires 279 respondents between the ages of 18 and 59 out of a total population of 675 selected randomly for this study. This value includes the addition of 10% for anticipated nonresponse case rates.

Questionnaire’s Development

A self-administered questionnaire adapted from a previous study by Rajamoorthy et al. (2022) was used to collect data from respondents. The instrument comprised three sections: demographic information, and knowledge and awareness of HFMD among parents. The survey was designed to be completed within approximately 10 minutes, based on preliminary testing with a small subset of participants. Each correct response was awarded a score of “1,” while incorrect responses scored “0.” A higher total score reflected greater knowledge and awareness of HFMD infection, calculated as the sum of all correct answers (Rajamoorthy et al., 2022).

Part A: Socio-demographic

The first section was the respondent’s demographic characteristics of the study population. There were eight questions on gender, age, level of education, occupation, race, marital status, household income, and the number of children. Each question had four answer choices, but respondents must only select one, except for the age query only needs to be filled in as usual.

Part B: Knowledge domain

For the second section, 47 items were used to assess the respondents' knowledge of the disease (Rajamoorthy et al., 2022). There were six different parts to the knowledge domain, with varying maximum totals for each. The possible responses to all the questions within the knowledge domain were "yes" or "no", and there were multiple answer choices for transmission, clinical features, prevention and treatment, severe signs, and management of HFMD.

Part C: Awareness domain

The third section, comprised of 14 items for awareness domains, was used to assess the awareness of the infected child, signs, and symptoms of severe complications of HFMD where only multiple answer choices were available, and each part had a different maximum total score (Rajamoorthy et al., 2022).

Ethical Approval

The human participant studies with reference number FERC/FSK/MR/2023/00071 were scrutinised and sanctioned by the Faculty Ethics Research Committee (FREC), Universiti Teknologi MARA, Cawangan Selangor, Puncak Alam Campus. The study was carried out after receiving approval from the committee regarding the research.

Statistical Analysis

Using SPSS version 23, socio-demographic data were analysed through descriptive analysis. Statistical Package for the Social Sciences (SPSS) version 28.0 software was used to analyse and interpret the obtained data. A descriptive analysis was performed on respondents' socio-demographic characteristics, knowledge, and awareness of HFMD. The variables such as gender, age, level of education, occupation, race, marital status, household income, and the number of children were summarized and then reported in frequency distribution and percentage. Numerical data of the total score of knowledge and awareness domains for each part, respectively, was calculated as mean and standard deviation, where each has a different maximum value.

Although the data did not satisfy the assumption of normality based on the Kolmogorov-Smirnov test ($p < 0.01$), a visual examination of the histograms revealed a distribution that approximated a normal curve. Therefore, the Central Limit Theorem was employed to justify the

normality assumption for subsequent analyses. Knowledge and awareness levels were classified as *good*, *moderate*, or *poor* using an 80% cut-off point, scores above 80% were considered good, 60–80% moderate, and below 60% poor, based on previous classifications by Charoenchokpanit and Pumpaibool (2013) and Harapan et al. (2018). A Pearson correlation was used to assess the relationship between total knowledge and awareness scores. One-way ANOVA was conducted to compare mean scores across different educational levels. Statistical significance was set at $p < 0.05$ (two-tailed) with a 95% confidence interval. All data were analyzed and presented in tables and figures.

RESULTS

Demographic Characteristics of Respondents

A total of 279 respondents participated in the study and were included in the statistical analysis. Their socio-demographic characteristics are summarized in Table 1.

Table 1: Socio-demographic of respondents ($n=279$)

Variables	Frequency, (n)	Percentage, (%)
Gender		
Male	186	66.7
Female	93	33.3
Age group		
18-29	107	38.4
30-39	59	21.1
40-49	57	20.4
50-59	56	20.1
Education		
High school or below	47	16.8
Certificate or diploma	110	39.4
Bachelor's degree	93	33.3
Postgraduate education	29	10.4
Occupation		
Public sector	68	24.4
Private sector	93	33.3
Self-employed	47	16.8
Others	71	25.4
Race		
Malay	278	99.6
Others	1	0.4
Marital status		
Married	225	80.6
Divorced/widow/widower	54	19.4
Household income		
Less than RM1000	35	12.5
RM1001-RM3000	97	34.8
RM3001-RM5000	88	31.5
RM5001-RM7000	38	13.6
RM7001-RM9000	16	5.7

RM9001 or above	5	1.8
Number of children		
1	96	34.4
2	67	24.0
3	52	18.6
4 and above	64	22.9

Distribution of Knowledge and Awareness of HFMD

Table 2 presents the classification of respondents' knowledge and awareness levels. Only 9.0% (n=25) demonstrated good knowledge of HFMD, while 29.7% had moderate knowledge. A majority—61.3% (n=171)—had poor knowledge, with most scoring below 50% on key items related to HFMD management and prevention. Awareness levels were similarly low: only 35.8% were categorized as having good awareness, while 42.7% demonstrated poor awareness, including limited recognition of symptoms and potential complications. These findings underscore the urgent need for targeted public health education in this community.

Table 2: Distribution of knowledge and awareness of HFMD (n=279)

Statement	Level of Knowledge and Awareness of HFMD					
	Low (<60%)		Moderate (60-80%)		High (>80%)	
	n	%	n	%	n	%
Knowledge score	171	61.3	83	29.7	25	9.0
Awareness score	119	42.7	60	21.5	100	35.8

Correlation Between Knowledge and Awareness

As shown in Table 3, a strong and statistically significant positive correlation was found between knowledge and awareness of HFMD ($r = 0.832$, $p < 0.001$), indicating that respondents with higher knowledge scores also had higher awareness levels.

Table 3: Correlation between knowledge and awareness of HFMD (n=279)

Variable	Awareness of HFMD
Knowledge of HFMD	0.832 ^a (<0.01) ^b

^a Pearson correlation coefficient

^b P-value

Comparison between mean total score knowledge and awareness of HFMD with respondents' educational background

Based on Table 4, a one-way ANOVA test was conducted. The P-value is not significant since $p > 0.05$. $F(3,275) = 1.971$, $p = 0.119$. Therefore, there was no significant difference in mean total score knowledge of HFMD among parents between different levels of educational background. It indicated that knowledge of HFMD was independent of the respondents' educational background levels.

Table 4: Comparison of mean total score knowledge of HFMD between different levels of educational background (n=279)

Variables	n	Mean Total Score Knowledge of HFMD (SD)	F-statistic ^a (df)	P-value
Level of educational background				
High educational or below	47	24.32 (9.59)	1.971 (3, 275)	0.119
Certificate or diploma	110	23.60 (8.50)		
Bachelor's degree	93	25.00 (7.49)		
Postgraduate education	29	24.81 (8.48)		

^a One-way ANOVA tests

According to Table 5, the result indicated no significant difference between awareness of HFMD and levels of educational background. Based on the one-way ANOVA test, the P-value was 0.127, $p > 0.05$, whereas $F(3,275) = 1.918$. The differences in mean between high school or below, certificate or diploma, bachelor's degree, and postgraduate education were not too large. It shows that a higher educational background does not influence the awareness of HFMD.

Table 5: Comparison of mean total score awareness of HFMD between different levels of educational background (n=279)

Variables	n	Mean Total Score Awareness of HFMD (SD)	F-statistic ^a (df)	P-value
-----------	---	---	-------------------------------	---------

Level of educational background					
High educational or below	47	8.68 (4.52)	1.918 (3, 275)	0.127	
Certificate or diploma	110	7.76 (4.05)			
Bachelor's degree	93	9.14 (4.17)			
Postgraduate education	29	8.28 (3.85)			

^a One-way ANOVA tests

DISCUSSION

Hand, Foot, and Mouth Disease (HFMD) is a viral illness predominantly affecting young children, with a disproportionate impact in rural areas. Understanding parental knowledge in such settings is vital for tailoring effective public health strategies. A notable 732.2% increase in HFMD cases was reported by the Terengganu State Health Department (JKNT), underscoring the urgency for intervention.

In this study, most respondents were male, in contrast to findings from Singapore where females were predominant (Wang & Pang, 2022). This may reflect sociocultural and demographic factors, including male dominance in community engagement and greater access to digital tools (Chakrabarti et al., 2019; Alkhamis et al., 2021). Conversely, women may face participation barriers such as limited access to devices and domestic responsibilities (Kim et al., 2020). Future surveys should address these disparities to improve gender representation. The predominance of younger participants likely reflects their greater digital literacy and access (Li et al., 2022).

Parental knowledge of HFMD etiology was limited, with frequent misclassification of symptoms as chickenpox, herpangina, or measles (Santos et al., 2019). This may stem from perceptions of HFMD as a mild illness and lack of formal health education (Chang et al., 2011). Delayed recognition increases the risk of complications such as nail loss, viral meningitis, and encephalitis (CDC, 2021; Sun et al., 2016).

Antibiotic misuse was prevalent, reflecting a misconception of antibiotics as a universal treatment (Mallah et al., 2020). Educational campaigns must emphasize the viral nature of HFMD and discourage

inappropriate antibiotic use, leveraging media, clinics, and community outreach.

Awareness of HFMD's risks to vulnerable populations, immunocompromised individuals, the elderly, and pregnant women was also lacking (Kaminska et al., 2013). Reliable information from agencies such as CDC, WHO, and MOH Malaysia is essential to address this gap (Li et al., 2020).

Knowledge of the fecal–oral transmission route was limited, likely due to infrequent outbreak exposure (Mohamed et al., 2020). Similar patterns were observed in Selangor (Qi et al., 2019), contrasting with Chinese studies where parents were more informed (CDC, 2023a). Promoting hygiene practices such as sanitizing surfaces remains essential (CDC, 2023b).

While parents recognized typical HFMD symptoms fever, ulcers, and blisters, awareness of severe signs such as gait disturbances was low. This is concerning given the potential for Enterovirus A71-related complications, including acute flaccid paralysis (Zainuddin, 2007; Apostol et al., 2019). NGOs and schools can strengthen awareness through workshops and curricular integration (Wang & Pang, 2022).

Most parents acknowledged the role of hygiene in HFMD prevention, aligning with findings in Selangor and elsewhere (Rajamoorthy et al., 2022; Wang et al., 2016). However, many incorrectly believed water alone sufficed for handwashing. Soap is necessary for removing virus particles (Guo et al., 2018), and evidence shows that soap use significantly reduces HFMD transmission, whereas water alone does not (Zhang et al., 2016). Misconceptions also surrounded alcohol-based sanitizers, which are ineffective against non-enveloped viruses like EV-A71 (Chang et al., 2013; Golin et al., 2020).

Although nutrition and hydration were identified as important for recovery, knowledge was inconsistent (Aman & Masood, 2020). Health professionals should leverage social media platforms to disseminate accurate, engaging dietary information.

A strong positive correlation ($r = 0.832$) was observed between knowledge and awareness. Better-informed parents demonstrated enhanced understanding of transmission, symptoms, and prevention. Nonetheless, overall knowledge and awareness remained suboptimal. In contrast, studies elsewhere reported moderate awareness and adherence to preventive behaviors such as disinfecting toys (Mansur & Ahmad, 2021; Rajamoorthy et

al., 2022). Low awareness may result from urban density, migrant populations (Wang et al., 2018), media access gaps, and cultural beliefs (Tran et al., 2021).

No significant association was found between parental education level and HFMD knowledge or awareness, corroborating previous findings (Mahadzar & Rahman, 2019; Nithya & Prakash, 2020). However, post-intervention improvements have been documented (Tran et al., 2021), emphasizing the potential of community-based educational initiatives (Kua & Pang, 2020). Supporting this, a study in Saudi Arabia found that only 31.6% of 260 medical students had adequate knowledge of HFMD, with higher scores among those previously educated on the disease (Alshahrani et al., 2024). These findings suggest that even individuals with higher educational backgrounds may benefit from structured health education, and underline the importance of accessible, targeted awareness efforts.

Cultural practices in rural areas, including irregular access to hygiene resources, reliance on traditional medicine, and attribution of illness to environmental factors, highlight the need for culturally appropriate interventions. Collaborative education programs led by MOH Malaysia, in partnership with NGOs, schools, and the media, should employ accessible platforms such as mobile apps and television broadcasts (Al Dmour et al., 2020). Rural health literacy disparities, shaped by limited access to healthcare and education, require targeted digital and community-based strategies (Health Policy Institute, 2023; Advent Health University, 2023). Engaging schools, community leaders, and healthcare workers enhances message credibility and reach (Smith et al., 2018). Social media platforms such as Facebook and WhatsApp also offer scalable channels for disseminating preventive messages (Chan et al., 2019).

Finally, parents with higher education levels or healthcare-related occupations demonstrated better HFMD knowledge, likely due to increased health information access (Smith et al., 2018; Tan et al., 2020). These findings underscore the need for targeted, equitable interventions to improve disease prevention across demographic groups.

CONCLUSION

Parents in Kampung Tuan Mandak showed limited knowledge and awareness of Hand, Foot, and Mouth Disease (HFMD), particularly regarding its causes, transmission, symptoms, treatment, and prevention. This lack of awareness was strongly associated with insufficient knowledge, and no significant link was found between

parental education level and awareness of the disease. These findings highlight the importance of strengthening health education efforts, especially in rural communities. Collaborative initiatives, through schools, community leaders, and healthcare providers, can help improve parental understanding of HFMD. Continued support and outreach from public health authorities will be valuable in enhancing awareness and encouraging effective prevention and control practices.

ACKNOWLEDGEMENT

We would like to express our sincere gratitude the Centre for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Selangor Branch, for the support and encouragement throughout this study. Our heartfelt thanks also go to the Faculty Research Ethics Committee (FREC) for their approval and guidance, and to Dr. Siti Nor Ismalina Isa for her invaluable assistance in statistical analysis. This study was conducted without any financial support.

REFERENCES

- Advent Health University. Improving healthcare in rural areas: addressing health literacy and access challenges. 2023. Available from: www.ahu.edu
- Al-Dmour H, Masa'deh R, Salman A, Abuhashesh M, Al-Dmour R. Influence of social media platforms on public health protection against the COVID-19 pandemic via the mediating effects of public health awareness and behavioral changes: integrated model. *J Med Internet Res.* 2020;22(8):e19996. <https://doi.org/10.2196/19996>
- Alkhamis A, Zaidan E, Bin Zayed M. Gender disparities in technology access in rural settings: a case study of mobile phone usage in the Middle East. *Technol Soc.* 2021;45(4):587–602. <https://doi.org/10.1080/2345678901>
- Alshahrani NZ, Bukhari SM, Bukhari LM, Alghamdi LS, Aljohani SR, Ghurab RA. Knowledge and attitudes of hand, foot, and mouth disease infection among medical students and physicians at the University of Jeddah. *Front Trop Dis.* 2024;5:1383211. doi:10.3389/fitd.2024.1383211
- Aman F, Masood S. How nutrition can help to fight against COVID-19 pandemic. *Pak J Med Sci.* 2020;36(COVID19-S4): S121–3.

- Apostol LN, Shimizu H, Suzuki A, Umami RN, Jiao MMA, Tandoc A, et al. Molecular characterization of enterovirus-A71 in children with acute flaccid paralysis in the Philippines. *BMC Infect Dis.* 2019;19(1):1.
- Centers for Disease Control and Prevention (CDC). Complications of Hand, Foot, and Mouth Disease. 2021. Available from: <https://www.cdc.gov/hand-foot-mouth/about/complications.html>
- Centers for Disease Control and Prevention (CDC). Prevent Hand, Foot, and Mouth Disease. 2023a. Available from: <http://www.cdc.gov/hand-foot-mouth/about/transmission.html>
- Centers for Disease Control and Prevention (CDC). Symptoms of Hand, Foot, and Mouth Disease. 2023b. Available from: <http://www.cdc.gov/hand-foot-mouth/about/transmission.html>
- Chakrabarti P, Lee L, Kuo Y. Access to digital technology and gender disparities in rural communities: a study of rural India. *J Rural Dev.* 2019;38(2):215–30. <https://doi.org/10.1080/1234567890>
- Chan L, Chen D, Wang S, Liu H. The impact of education on health literacy and preventive health behaviors. *Int J Public Health.* 2019;64(3):225–35.
- Chang AC, Jacobsen KH, Lin KW, Teng L. Enterovirus knowledge and handwashing practices among nurses in a hospital in Taipei, Taiwan. *Taiwan Epidemiol Bull.* 2011;27(6):81–101.
- Chang SC, Chen YC, Fang CT, Hung CC, Chang SY. Efficacy of alcohols and alcohol-based hand disinfectants against human enterovirus 71. *J Hosp Infect.* 2013;83(4):288–93. <https://doi.org/10.1016/j.jhin.2012.12.010>
- Charoenchokpanit R, Pumpaibool T. Knowledge, attitude and preventive behaviors towards Hand Foot and Mouth Disease among caregivers of children under five years old in Bangkok, Thailand. *J Health Res.* 2013;27(5):281–6. Available from: <https://www.tci-thaijo.org/index.php/jhealthres/article/view/88692>
- Golin AP, Choi D, Ghahary A. Hand sanitizers: a review of ingredients, mechanisms of action, modes of delivery, and efficacy against coronaviruses. *Am J Infect Control.* 2020;48(9):1062–7. <https://doi.org/10.1016/j.ajic.2020.06.182>
- Guo N, Li J, Li H, Hao Y, Guo J, Zhang N, et al. Effect of hand washing and personal hygiene on Hand Foot Mouth Disease: a community intervention study. *Medicine (Baltimore).* 2018;97(51):e13144. <https://doi.org/10.1097/MD.00000000000013144>
- Harapan H, Rajamoorthy Y, Anwar S, Bustamam A, Radiansyah A, Angraini P, et al. Knowledge, attitude, and practice regarding dengue virus infection among inhabitants of Aceh, Indonesia: a cross-sectional study. *BMC Infect Dis.* 2018;18(1):96. <https://doi.org/10.1186/s12879-018-3006-z>
- Health Policy Institute. Rural and urban health: healthcare disparities and challenges. Georgetown University. 2023. Available from: <https://hpi.georgetown.edu>
- Ismail N, Tan JH, Lee CT. Health disparities between rural and urban populations in Malaysia: a literature review. *Asian Pac J Public Health.* 2018;30(5):428–36.
- Ismail NA, Zahiruddin WM, Fuzi NMHM. Risk factors of Hand, Foot and Mouth Disease (HFMD) outbreak cases among children under five years old in a northeastern state of Peninsular Malaysia. *Malays J Public Health Med.* 2021;21(2):315–20. <https://doi.org/10.37268/mjphm/vol.21/no.2/art.1028>
- Kaminska K, Martinetti M, Lucchini R, Filippi A, Mainetti C. Coxsackievirus A6 and Hand, Foot and Mouth Disease: three case reports of familial child-to-immunocompetent adult transmission and a literature review. *Case Rep Dermatol.* 2013;5(2):203–9. <https://doi.org/10.1159/000354533>
- Kim H, Jeong J, Lee Y. Gender differences in participation in online surveys and digital platforms in rural communities. *J Rural Sociol.* 2020;25(1):88–103. <https://doi.org/10.1080/1122334456>
- Koh WM, Bogich T, Siegel K, Jin J, Chong EY, Tan CY, et al. The epidemiology of Hand, Foot and Mouth Disease in Asia: a systematic review and analysis. *Pediatr Infect Dis J.* 2016;35(10):e285–300. <https://doi.org/10.1097/INF.0000000000001009>
- Kua JA, Pang J. The epidemiological risk factors of Hand, Foot, Mouth Disease among children in Singapore: a retrospective case-control study. *PLoS One.* 2020;15(8):e0236711. <https://doi.org/10.1371/journal.pone.0236711>

- Li W, Zhu Y, Zhang X. Impacts social media have on young generation and older adults. In: Proceedings of ICHESS2021. 2022;615:294–300. <https://doi.org/10.2991/assehr.k.211220.051>
- Li W, Cui H, Wang Y, Zhang Q, Xu Y. Public health education for parents during the outbreak of COVID-19: a rapid review. *Ann Transl Med.* 2020;8(10):628. <https://doi.org/10.21037/atm-20-3312>
- Li Y, Wang C, Chen Q, Cheng H, Lu W, Zhao H, et al. Effectiveness of EV-A71 vaccination in prevention of pediatric HFMD associated with EV-A71 virus infection requiring hospitalization. *Lancet Child Adolesc Health.* 2019;3(10):697–704. [https://doi.org/10.1016/S2352-4642\(19\)30185-3](https://doi.org/10.1016/S2352-4642(19)30185-3)
- Mahadzar SAS, Rahman HA. Knowledge, attitude and practice towards Hand, Foot and Mouth Disease among nursery governesses in Klang Valley. *Malays J Med Health Sci.* 2019;15(SP4):40–7.
- Mallah N, Badro DA, Younes S, Itani M, Soubra R, Khansa A, et al. Association of knowledge and beliefs with the misuse of antibiotics in parents: a study in Beirut (Lebanon). *PLoS One.* 2020;15(7):e0232464. <https://doi.org/10.1371/journal.pone.0232464>
- Mansur NNH, Ahmad A. Knowledge and prevention practices of HFMD among parents and caregivers in Malaysia. *Malays J Public Health Med.* 2021;21(1):29–36. <https://doi.org/10.37268/mjphm/vol.21/no.1/art.485>
- Ministry of Health Malaysia. Hand, foot, and mouth disease cases surge by 15-fold by mid-May 2022 compared to 2021. *Malaysia Now.* 2022. Available from: <https://www.malaysianow.com>
- Ministry of Health Malaysia. HFMD cases rise with highest numbers in urbanized states. *Harakah Daily.* 2022. Available from: <https://harakahdaily.net>
- Mohamed NA, Ismail A, Ahmad N, Salleh N. HFMD educational workshop for preschool teachers: an interventional study. *Int J Res Pharm Sci.* 2020;11(SPL4):1551–8. <https://doi.org/10.26452/ijrps.v11ispl4.4337>
- Nithya S, Prakash S. Access of knowledge on HFMD among the mothers of under-five children and nurses at pediatric wards. *Int J Contemp Pediatr.* 2020;13(3). <https://doi.org/10.5005/jp-journals-10084-12164>
- Noor A, Remo O. Health literacy in rural and urban populations: a systemic review. *Patient Educ Couns.* 2020;103(10):2142–54. <https://doi.org/10.1016/j.pec.2020.06.007>
- World Health Organization. Outbreak of Hand, Foot and Mouth Disease: Malaysia. *Wkly Epidemiol Rec.* 1997;72(28):197–202. Available from: <https://apps.who.int/iris/handle/10665/230186>
- Puenpa J, Vongpunsawad S, Phuektes P, Poovorawan Y. The history of Enterovirus A71 outbreaks and molecular epidemiology in Asia-Pacific. *J Biomed Sci.* 2019;26(1):75. <https://doi.org/10.1186/s12929-019-0573-2>
- Qi L, Cheng X, Zhang L, Tan X, Liu J, Xu W, et al. Enterovirus 71 vaccine acceptance among parents of children <5 years old in Chongqing. *PLoS One.* 2019;14(11):e0225569. <https://doi.org/10.1371/journal.pone.0225569>
- Rajamoorthy Y, Ahmad A, Mohamed NM. Preventive behaviors among parents towards HFMD in Selangor using Health Belief Model. *IJUM Med J Malays.* 2022;21(1):114–21. <https://doi.org/10.31436/imjm.v21i1.1975>
- Santos JA, De Leon A, Valencia S, Reyes J. The confusion between HFMD and other childhood rashes. *Int J Pediatr Health.* 2019;31(6):407–15. <https://doi.org/10.1002/ijph.2859>
- Smith J, Thomson M, Lee R. Health literacy and disease prevention: the role of education. *J Health Educ Res.* 2018;34(2):99–112.
- Sun L, Wang Y, Song L, Liu J, Wang L, Zhang Y. Evaluating the transmission routes of HFMD in Guangdong, China. *Am J Infect Control.* 2016;44(2):e13–4. <https://doi.org/10.1016/j.ajic.2015.04.202>
- Tan Y, Ong B, Hashim R. Healthcare occupation and knowledge of infectious diseases: awareness of HFMD in Malaysia. *J Infect Dis.* 2020;56(4):112–21.
- Tran HD, Doan HT, Nguyen QT, Nguyen TL, Le DN, Nguyen TP. Effectiveness of educational communication in knowledge, attitude, and practice to HFMD prevention. *J Med Assoc Thai.* 2021;104(8):1229–34. <https://doi.org/10.35755/jmedassth.2021.08.10816>

- Wang J, Xiao Y, Cheke RA. Modelling the effects of contaminated environments on HFMD. *BioSystems*. 2016;140:1–7.
<https://doi.org/10.1016/j.biosystems.2015.12.001>
- Wang MX, Pang J. KAP of HFMD prevention amidst COVID-19: a cross-sectional study. *Front Public Health*. 2022;10:908004.
<https://doi.org/10.3389/fpubh.2022.908004>
- Wang XF, Guo N, Du ZL, Qian H, Chen D, Li Y, et al. Epidemiological features of HFMD among Chinese preschool children. *Iran J Public Health*. 2018;47(9):1234–43.
- World Health Organization. WHO guideline on health workforce development. 2021. Available from: <https://www.who.int/publications-detail/health-workforce-development>
- Zainuddin AW. Hand Foot and Mouth Disease (HFMD) Guidelines. Ministry of Health Malaysia; 2007.
- Zhang D, Zhang J, Ma X, Di B, Shi W, Wang Y. Hand-washing: the main strategy for avoiding HFMD. *Int J Environ Res Public Health*. 2016;13(6):606.
<https://doi.org/10.3390/ijerph13060606>

The Influence of Social Media Addiction on Body Dissatisfaction, Physical Activity, and Eating Behaviour Among Undergraduate Health Sciences and Uitm Puncak Alam

Siti Maisarah Harun¹ and Mazura Bahari^{1,2*}

¹Center for Medical Laboratory Technology Studies, Faculty of Health Sciences, Universiti Teknologi MARA, Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

²Research and Education in Biomedical, Epidemiology and Immunology Aliances (REBEIA), FSK, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Background: Social media platforms, often dominated by idealized images from influencers and celebrities, can promote unrealistic standards and upward social comparison. **Methods:** This study investigates gender differences in social media addiction, body dissatisfaction, physical activity, and eating behaviors among 209 university students at UiTM Puncak Alam, with a slight female majority (53.4%). **Results:** Using a cross-sectional design and online questionnaire, the findings reveal that female students, despite being less physically active, report higher levels of social media addiction and body dissatisfaction compared to males. Specifically, 74.8% of females and 72.2% of males were classified as addicted to social media, with females scoring higher in both addiction and body dissatisfaction. In contrast, 96.9% of males were classified as physically active, compared to 65.8% of females. Correlation analyses indicated that social media addiction was positively associated with body dissatisfaction ($r = 0.332$), enjoyment of food ($r = 0.391$), and emotional undereating ($r = 0.358$), and negatively associated with physical activity ($p = -0.204$). Multiple regression analysis showed that body dissatisfaction ($B = 0.146$, $p < 0.001$), enjoyment of food ($B = 1.624$, $p = 0.010$), and physical activity ($B = -0.035$, $p = 0.009$) were significant predictors of social media addiction, explaining 27.5% of its variance ($R^2 = 0.275$). No significant gender differences were found in eating behaviors such as enjoyment of food and emotional undereating. **Conclusion:** These findings suggest that excessive social media use may contribute to increased body dissatisfaction and decreased physical activity, particularly among female students. Targeted interventions to manage social media engagement and promote body positivity could support healthier lifestyle habits in university populations.

Keywords:

body dissatisfaction; physical activity; eating behaviour; gender differences; social media addiction

INTRODUCTION

Students in the health sciences are uniquely positioned as future healthcare professionals, for whom maintaining a healthy appearance and lifestyle is both a personal responsibility and a professional expectation. This occupational standard may heighten their sensitivity to health, fitness, and body image-related content on social media, thereby increasing their susceptibility to its potential negative impacts compared to students from non-health-related disciplines.

Social media refers to websites and applications that enable users to communicate and share information via computers or mobile devices. It has transformed communication by fostering global connections through platforms such as Instagram, Facebook, TikTok, Twitter, and Snapchat. These platforms support social interaction

by allowing users to maintain communities, share permanent content, and post temporary “stories” that disappear after 24 hours (Kinkel-Ram et al., 2022). Social media serves multiple purposes, including entertainment, social engagement, and information sharing. However, its effects are complex and depend on factors such as frequency, duration, and usage patterns. Excessive or unhealthy use has been associated with negative outcomes (Amor, 2024), including body image concerns and disordered eating behaviors (Kinkel-Ram et al., 2022). Among young adults, especially women, social media particularly image, centric platforms like Instagram, amplifies exposure to idealized body standards. Influencers and celebrities often present curated images that portray unrealistic beauty and lifestyle ideals (Wang et al., 2023). This exposure can trigger upward social comparison, where users evaluate themselves against

* Corresponding author.

E-mail address: mazurabahari@uitm.edu.my

these unattainable standards, often resulting in body dissatisfaction, defined as negative thoughts and feelings about one's body (Amor, 2024; Pedalino & Camerini, 2022). Women are more likely than men to engage in such comparisons, which can influence eating behaviors and food-related attitudes. Frequent exposure to images of thin bodies and low-calorie meals may contribute to body image disturbances and disordered eating. Notably, increased Instagram use has been linked to symptoms of orthorexia nervosa, characterized by rigid, health-obsessed dietary behaviors influenced by online trends and associated with neurological changes related to self-control. Despite these risks, social media can also have positive effects. Exposure to body-positive content has been shown to enhance body satisfaction and emotional well-being among university students (Griffin et al., 2022). Additionally, social media can support healthy lifestyle adoption by promoting motivational content related to exercise and nutrition (Krupa-Kotara et al., 2023). This study is grounded in Social Comparison Theory, which suggests that individuals evaluate themselves by comparing with others, particularly when objective standards are lacking. On social media, these comparisons often involve idealized images that can negatively influence self-image and behavior. Additionally, the Uses and Gratifications Theory supports this research by proposing that individuals engage with media to fulfill specific needs, such as seeking validation, information, or social connection, which can shape their health-related behaviors. Based on these theoretical frameworks, the study hypothesizes that: (1) higher levels of social media addiction are positively associated with body dissatisfaction and disordered eating behaviours, and (2) social media addiction is negatively associated with physical activity levels. Despite growing interest in this area, the specific relationships between social media addiction, body dissatisfaction, physical activity, and eating behaviours among health science students remain underexplored. Undergraduate health science students face distinct challenges, including demanding academic and clinical workloads, which may intensify the impact of social media use on their health behaviours. While existing research highlights both positive and negative effects of social media, little is known about how it specifically influences body image and lifestyle habits in this population. This study aims to address this gap by investigating these associations among students at UiTM Puncak Alam.

MATERIALS AND METHODS

A cross-sectional study design was employed to examine the influence of social media on body dissatisfaction,

physical activity, and eating behaviors among students. Data were collected through an online questionnaire distributed via Google Forms and shared across social media platforms. The survey included validated scales and items assessing social media addiction, body dissatisfaction, physical activity, and eating behaviors.

Sample Design and Setting

The survey was conducted over a three-month period, from March to May 2024, following approval from the Research Ethics Committee. Data collection was carried out using an online questionnaire administered through Google Forms. The survey link and corresponding QR code were disseminated via various social media platforms, including Facebook, Instagram, Telegram, and WhatsApp. Participants were fully informed that their participation was voluntary, and no compensation was offered. Responses were automatically recorded upon completion of the questionnaire.

Sample Selection

Participants were recruited through convenience sampling among students from the Faculty of Health Sciences via online distribution. To be eligible, participants were required to have a good command of the English language, be between 18 and 30 years of age, and be frequent users of social media. This age group was selected because adolescents and young adults who regularly engage with social media are more likely to experience body image concerns. Exclusion criteria included individuals who did not meet the inclusion criteria, were not enrolled in the Faculty of Health Sciences at UiTM Puncak Alam, had limited English proficiency, or were over the age of 30.

Sample size

The sample size was determined to meet the minimum requirement for achieving statistical significance, ensuring that the results would be accurate and representative of the target population. A previous study by Salari et al. (2023) reported a pooled global prevalence of social media addiction of 18.4% among university students. Accordingly, this study used the same proportion (18.4%) to estimate the population parameter for sample size calculation.

$$n = \frac{Z^2 [P(1-p)]}{e^2}$$

$$n = \frac{(1.96)^2 [0.184(1-0.184)]}{(0.06)^2}$$

$$n = 160$$

n = sample size

- Z = the z-score, which is a measure of how confident study want to be in study result (1.96 for a 95% confidence level)
- P = the estimated proportion population
- e = the amount of error study will tolerate in study results (0.06 for a 6% margin of error)

The aim of this study was to achieve a 95% confidence level with a 6% margin of error (e). Accordingly, a minimum sample size of 160 respondents was required to ensure that the results would be accurate within this margin at the specified confidence level.

Questionnaire Development

The study utilized an online questionnaire to collect data from respondents. The questionnaire was divided into five sections: Part A covered demographic information; Part B included the Bergen Social Media Addiction Scale (BSMAS); Part C featured the 16-item Body Shape Questionnaire (BSQ-16); Part D comprised the Godin Leisure-Time Exercise Questionnaire (GLTEQ); and Part E contained the Adult Eating Behaviors Questionnaire.

Part A: General Information

The General Information section of the questionnaire aimed to collect basic demographic and physical data from respondents. Part A included questions on gender, age, course of study, current weight, and height. Most questions provided multiple-choice options with single-answer selection, except for age, weight, and height, which required participants to provide written responses.

Part B: Bergen Social Media Addiction Scale (BSMAS)

To assess the level of social media addiction, this study utilized the Bergen Social Media Addiction Scale (BSMAS), which comprises six items referencing experiences over the past year. Participants responded on a 5-point Likert scale, measuring the frequency of behaviors associated with excessive social media use. The total score indicated the degree of addiction, with higher scores reflecting greater usage. A score above 16 was considered indicative of social media addiction. Additionally, participants who answered “often” or “very often” to at least four items were categorized as addicted (O’Reilly & Richardson, 2018). The BSMAS has been validated in previous research and is adapted from the Bergen Facebook Addiction Scale, demonstrating strong reliability and construct validity (O’Reilly & Richardson, 2018) (Table 1).

Table 1: Interpretation score of BSMAS

Category	Inclusion
Not addicted	<16
Addicted	>16

Part C: Body Shape Questionnaire-16 Item (BSQ16) Version

The Body Shape Questionnaire (BSQ) is a self-report instrument designed to assess concerns related to body shape and weight. The original BSQ consists of 34 items and typically takes approximately 10 minutes to complete. A shortened version, the BSQ-16, offers a more efficient alternative while maintaining strong psychometric properties. The BSQ-16 focuses on participants’ body-related perceptions over the past four weeks. One version of the questionnaire uses a 6-point Likert scale, ranging from 6 (Never) to 1 (Always). Scores are categorized into four levels of concern: low concern (score < 38), mild concern (38–51), moderate concern (52–66), and marked concern (score > 66). Higher scores indicate greater levels of body dissatisfaction (Table 2). These classifications align with the corresponding score ranges on the BSQ, facilitating straightforward interpretation of results. Additionally, to approximate scores on the original BSQ-34, total scores from the BSQ-16 may be multiplied by a factor of 34/16. However, this is considered a rough estimation and should be interpreted with caution (O’Reilly & Richardson, 2018).

Table 2: Interpretation score of BSQ16

Category	Score
No concern	<38
Mild concern	38 – 51
Moderate concern	52 – 66
Highly concern	> 66

Part D: Godin Leisure-Time (GLT) Questionnaire

This study used a questionnaire to assess participants’ physical activity levels. It included four questions: the first three asked how many times in the past week participants engaged in strenuous, moderate, and light exercise. These were scored using the formula: (9 × strenuous) + (5 × moderate) + (3 × light), based on O’Reilly and Richardson (2018). The fourth question asked how often participants engaged in regular physical activity, with options of

"often," "sometimes," or "never." Based on their total score, participants were classified as active (≥ 24 units), moderately active (14–23 units), or insufficiently active/sedentary (< 14 units), as shown in Table 3.

Table 3: Interpretation score of GLT

Category	Score
Insufficient active	<14
Moderate active	14 – 23
Highly active	>23

Part E: Adult Eating Behaviour (AEB) Questionnaire

This study utilized an adapted version of the Child Eating Behavior Questionnaire (CEBQ) to assess participants' eating habits and attitudes. Originally developed to measure appetitive traits in children, the scale was modified for adult use (O'Reily & Richardson, 2018). It consisted of 35 items rated on a 5-point Likert scale, where participants selected the response that best described their typical behavior. Items included statements such as "I love food," with responses ranging from strong disagreement to strong agreement. The scale assessed eight dimensions of appetitive traits: hunger, food responsiveness, emotional overeating, enjoyment of food, satiety responsiveness, emotional undereating, food fussiness, and slowness in eating. For the purpose of this study, two traits were selected to represent contrasting eating behaviors: *enjoyment of food* (positive) and *emotional undereating* (negative).

Ethics Approval from The Committee

The title and protocol of this study were approved by the University Research Ethics Committee, under the reference number FERC/FSK/MR/2024/00098.

Statistical Analysis

The questionnaires will assess various aspects, including social media use, eating and exercise habits, and body image satisfaction. The collected data will be entered into SPSS for analysis. The initial phase will involve descriptive statistics to develop a general profile of the participants.

Descriptive Statistics

The initial stage of the analysis involved performing descriptive statistics to establish a general profile of the participants. This provided a comprehensive overview of the sample characteristics, including measures such as means, standard deviations, frequencies, and

percentages. This step was essential for understanding the demographic and behavioral patterns of undergraduate Health Sciences students at UiTM Puncak Alam. By summarizing the data, the descriptive analysis laid the foundation for subsequent inferential statistical procedures.

Independent T-Test

To examine if there were significant differences in social media addiction, body dissatisfaction, physical activity, and eating behavior between genders, independent t-tests were conducted. The independent t-test was a statistical method used to compare the means of two independent groups to determine if there was a statistically significant difference between them. The basic formula (Equation 1) for independent t-test as below;

$$t = \frac{(\bar{x}_1 - \bar{x}_2)}{S_{\bar{x}_1 - \bar{x}_2}} \tag{1}$$

In this formula, the numerator $(\bar{x}_1 - \bar{x}_2)$ represented the difference between the sample means, while the denominator $S_{\bar{x}_1 - \bar{x}_2}$ is the standard error of the different between the means. This t-value is compared against a critical value from the t-distribution table. If the t-value exceeds the critical value, the difference between the means is considered statistically significant, indicating that the observed difference is unlikely to have occurred by random chance. This test helped in identifying any gender-specific differences in the dependent variables, providing insights into how these behaviors and perceptions varied between male and female students.

Mann U-Whitney Test

The Mann-Whitney U test is a non-parametric statistical method used to compare differences between two independent groups on a single continuous or ordinal dependent variable. Unlike the independent t-test, which assumes that the data are normally distributed and have equal variances, the Mann-Whitney U test does not require these assumptions. The Mann-Whitney U test works by ranking all the values from both groups together in ascending order and then comparing the ranks. The test statistic, U, is calculated based on the sum of ranks for each group.

Correlation Coefficient

To investigate the relationships between social media addiction, body dissatisfaction, physical activity, and eating behavior, correlation coefficient analysis was employed. The correlation coefficient measured the strength and direction of the linear relationship between two continuous variables. The strength of the relationship

was indicated by the absolute value of the correlation coefficient, where values close to 1 or -1 signified a strong relationship, values around 0.5 signified a moderate relationship, and values close to 0 signified a weak or no relationship. The direction was indicated by the sign of the coefficient. A positive value meant that as one variable increased, the other also increased (positive correlation), while a negative value meant that as one variable increased, the other decreased (negative correlation). This analysis helped in identifying whether an increase or decrease in one variable was associated with an increase or decrease in another variable, thereby providing a clearer understanding of the interplay between these factors among the students.

Linear Regression

To determine if body dissatisfaction, physical activity, and eating behavior could predict social media addiction, linear regression analysis was utilized. Linear regression was a statistical technique that modelled the relationship between a dependent variable and one or more independent variables.

The simple linear regression formula (Equation 2) expressed as:

$$Y = B_0 + B_1X_i + \varepsilon_i \quad (2)$$

In this formula, Y represents the dependent variable we aim to predict, and X_i represents the independent variable. The term B_0 is the y-intercept, which is the value of Y when X_i is zero. The coefficient B_1 represents the slope of the regression line, indicating how much Y changes for a one-unit change in X_i . The term ε_i represents the error term, accounting for the variability in Y that cannot be explained by the linear relationship with X_i . This analysis helped in identifying the key predictors of social media addiction and informed the development of targeted interventions to address this issue among the students.

RESULTS

A total of 209 undergraduate Health Sciences students participated in the study, with slightly more females (53.4%) than males (46.6%). The average age was 23 years for both genders. Males were generally taller and heavier, while females had a higher percentage of underweight (28.8%) and obese (9.9%) individuals compared to males (20.6% and 3.1%, respectively). Social media addiction scores were higher among females (mean = 19.67) than males (mean = 18.36), with 74.8% of females and 72.2% of

males meeting the criteria for addiction. Body dissatisfaction was also slightly higher in females (mean = 25.22) than in males (mean = 22.99), although most participants showed low concern overall. Physical activity levels showed a clear gender difference. Males had higher activity scores (mean = 48.59) and a greater proportion were classified as active (96.9%) compared to females (mean = 32.50; active = 65.8%). Enjoyment of food and emotional under-eating scores were similar between genders, with females showing slightly higher averages. These results highlight notable gender differences in physical activity, body image, and social media use among the students.

Table 4: Descriptive Statistics

Variables	Total (N=208)	Male (n=97)	Female (n=111)
Age		23 (1.77)	23 (2.13)
Height (in cm)		174.22 (9.13)	161.87 (9.70)
BMMI		21.07 (3.75)	21.75 (4.48)
BMI category			
Underweight	52	20 (20.6%)	32 (28.8%)
Normal			
Overweight	119	63 (64.9%)	56 (50.5%)
Obese	23	11 (11.3%)	12 (10.8%)
	14	3 (3.1%)	11 (9.9%)
Social Media Addiction Score		18.36 (3.59)	19.67 (4.18)
Social Media Addiction			
Not Addicted	55	27 (27.8%)	28 (25.2%)
Addicted	153	70 (72.2%)	83 (74.8%)
Body Dissatisfaction Score		22.99 (5.29)	25.22 (7.61)
Body Dissatisfaction level			
Low concern	200	96 (99.0%)	104 (93.7%)
Mild concern	8	1 (1.0%)	8 (6.3%)
Moderate concern	0	0 (0%)	0 (0%)
Physical Activity Score		48.59 (15.24)	32.50 (17.63)
Physical Activity Level			
Insufficient	16	0 (0%)	16 (14.4%)
Active			
Moderate	25	3 (3.1%)	22 (19.8%)
Active			
Active	167	94 (96.9%)	73 (65.8%)

Enjoyment of Food Score	3.12 (0.56)	3.21 (0.51)
Emotional Under-eating Score	2.91 (0.62)	2.95 (0.63)
Mean (SD; n (%))		

Inferential Statistics

Objective one was to examine the significant differences in social media addiction, body dissatisfaction, physical activity, and eating behavior among genders. Preliminary assumption testing was conducted to check normality before deciding on parametric or non-parametric statistics. The Kolmogorov-Smirnov test indicated that social media addiction and emotional under-eating were not normally distributed for both genders. Body dissatisfaction was normally distributed for males but not for females, while the enjoyment of food was not normally distributed for males but was approximately normally distributed for females. Physical activity was normally distributed for both genders. Given these mixed results, an independent t-test was used for normally distributed data, while the Mann-Whitney U test was used for non-normally distributed data. In cases where the normality test results showed that data for one group was normally distributed and the data for the other group was not normally distributed, we considered using a non-parametric test.

An independent t-test was conducted to examine if there was a significant difference in physical activity between genders among undergraduate health sciences students at UiTM Puncak Alam. Table 5 showed that the mean difference in physical activity between genders was statistically significant, with females (mean = 32.39, SD= 17.59) being significantly less active than males (mean = 48.59, SD = 15.24).

Table 5: Independent T-Test

Variable	Male (n=97) Mean (SD)	Female (n=110) Mean (SD)	Mean diff. (95% CI)	t-stats (df)	P-value
Physical Activity	48.59 (15.24)	32.39 (17.59)	16.20 (11.67, 20.73)	7.05 (206)	<0.001

The Mann-Whitney U test was conducted to examine if there were significant differences in social media addiction, body dissatisfaction, enjoyment of food, and emotional under-eating between genders. Table 6 indicated significant differences between genders in social

media addiction and body dissatisfaction among undergraduate health sciences students at UiTM Puncak Alam. Specifically, females reported significantly higher levels of social media addiction ($U = 4439.0$, $p = 0.029$) and body dissatisfaction ($U = 4258.0$, $p = 0.009$) compared to males. Conversely, males reported significantly higher levels of physical activity ($U = 2696.0$, $p < 0.001$) compared to females. However, no significant differences were found between genders in enjoyment of food ($U = 4783.5$, $p = 0.166$) and emotional under-eating ($U = 5073.5$, $p = 0.543$). These findings suggested gender-specific patterns in social media usage, body image, and physical activity, but not in eating behaviors.

Table 6: Mann-Whitney U

Variables	Mean Rank		Mann-Whitney U	Z-score	P-value
	Male (n = 97)	Female (n = 110)			
Media Social Addiction	94.76	113.01	4439.0	-2.190	0.029*
Body Dissatisfaction	92.90	114.64	4258.0	-2.599	0.009*
Enjoyment of Food	98.31	109.91	4783.5	-1.387	0.166
Emotional Under-Eating	101.30	106.38	5073.5	-0.608	0.543

*Significance level = $p < 0.05$

Objective two was to examine the correlation between social media addiction, body dissatisfaction, physical activity, and eating behavior among genders. Preliminary assumption testing was conducted to check normality before deciding on parametric or non-parametric statistics. Scatter plots were used to assess whether the data exhibited a bivariate normal distribution. A bivariate normal distribution was indicated by a linear pattern in the scatter plots and an elliptical shape of the data points. For relationships that exhibited bivariate normal distribution, Pearson correlation was considered. For relationships that likely departed from bivariate normality, Spearman correlation was considered.

Table 7 presents the Pearson correlation coefficients among social media addiction, body dissatisfaction, physical activity, enjoyment of food, and emotional under-eating. Social media addiction showed significant and fair positive correlations with body dissatisfaction ($r = 0.332$), enjoyment of food ($r = 0.391$), and emotional under-eating ($r = 0.358$), indicating that higher social media addiction was associated with

greater body dissatisfaction, increased enjoyment of food, and higher emotional under-eating. A strong positive correlation was also found between enjoyment of food and emotional under-eating ($r = 0.694$), suggesting that those who reported greater enjoyment of food were more likely to engage in emotional under-eating. Additionally, body dissatisfaction showed weak but significant positive correlations with enjoyment of food ($r = 0.195$) and emotional under-eating ($r = 0.144$).

Table 7: Pearson Correlation between Social Media Addiction, Body Dissatisfaction, Physical Activity, and Eating Behaviour

Variables	Social Media Addiction	Body Dissatisfaction	Physical Activity	Enjoyment of Food	Emotional Under-Eating
Social Media Addiction	1				
Body Dissatisfaction	0.332**	1			
Physical Activity	-0.206**	-0.106	1		
Enjoyment of Food	0.391**	0.195**	-0.060	1	
Emotional Under-Eating	0.358**	0.144*	-0.034	0.694**	1

** Correlation is significant at the 0.001 level (2-tailed)

*Correlation is significant at the 0.005 level (2-tailed)

Table 8 presents the Spearman's rank correlation analysis. Social media addiction showed a weak but significant negative correlation with physical activity ($\rho = -0.204$), indicating that higher levels of social media addiction were associated with lower physical activity. Similarly, body dissatisfaction was weakly and negatively correlated with physical activity ($\rho = -0.205$), suggesting that greater body dissatisfaction was linked to reduced physical activity levels. No significant correlations were found between physical activity and either enjoyment of food ($\rho = -0.072$) or emotional under-eating ($\rho = -0.084$), indicating no meaningful association between these variables.

A multiple linear regression analysis was conducted to determine whether body dissatisfaction, physical activity, and eating behavior predicted social media addiction among undergraduate health sciences students at UiTM Puncak Alam (Table 9). The model accounted for approximately 27.5% of the variance in social media addiction ($R^2 = 0.275$), and this was statistically significant ($p < 0.001$). Body dissatisfaction ($B = 0.146$, $p < 0.001$) and enjoyment of food ($B = 1.624$, $p = 0.010$) emerged as significant positive predictors, indicating that higher levels

of both were associated with greater social media addiction. In contrast, physical activity ($B = -0.035$, $p = 0.009$) was a significant negative predictor, suggesting that increased physical activity was linked to lower social media addiction. Emotional under-eating ($B = 1.044$, $p = 0.052$) did not reach statistical significance. The regression analysis revealed that body dissatisfaction and enjoyment of food were significant positive predictors of social media addiction, while physical activity was a significant negative predictor. Emotional under-eating did not reach statistical significance but showed a potential positive trend.

Table 8: Spearman Correlation between Social Media Addiction, Body Dissatisfaction, Physical Activity, and Eating Behavior

Variables	Social Media Addiction	Body Dissatisfaction	Physical Activity	Enjoyment of Food	Emotional Under-Eating
Social Media Addiction	1				
Body Dissatisfaction	0.321**	1			
Physical Activity	-0.204**	-0.205**	1		
Enjoyment of Food	0.335**	0.134	-0.060	1	
Emotional Under-Eating	0.327**	0.070	-0.034	0.682**	1

** Correlation is significant at the 0.001 level (2-tailed)

Table 9: Linear Regression of Body Dissatisfaction, Physical Activity, and Eating Behavior Predicting Social Media Addiction

	R	adjR ²	B	t-stat	P-value
Model	0.507 ^a	0.275			<0.001**
Body Dissatisfaction			0.146 (0.074, 0.218)	4.000	<0.00*
Physical Activity			-0.035 (-0.061, -0.009)	-2.641	0.009*
Enjoyment of Food			1.624 (0.385, 2.863)	2.584	0.010*
Emotional Under-Eating			1.044 (-0.010, 2.098)	1.953	0.052

^aPredictors: (Constant): Emotional Under-eating, Physical Activity, Body Dissatisfaction, Enjoyment of Food

*Significance level, $p < 0.05$

DISCUSSION

This study examined gender differences in social media addiction, body dissatisfaction, physical activity, and

eating behaviors among undergraduate Health Sciences students at UiTM Puncak Alam. Health Sciences students may be particularly vulnerable to social media's effects due to professional expectations surrounding health, fitness, and appearance. These pressures, combined with academic stress, may encourage coping strategies involving social media, especially among female students.

Findings revealed that female students reported significantly higher levels of social media addiction and body dissatisfaction, aligning with previous research (Alfaya et al., 2023). However, the literature remains mixed, with some studies showing no gender differences (Ahmed et al., 2021; Mahmood et al., 2022) and others indicating higher addiction levels in males (Xuan & Amat, 2021). These inconsistencies may reflect gender-specific social media usage patterns, where females tend to use platforms for communication and comparison, while males use them for entertainment and networking (Aslan & Yaşar, 2020).

Female students also reported significantly greater body dissatisfaction, supporting past findings that females experience more appearance-related concerns (Manap et al., 2023). In contrast, male students were significantly more physically active, consistent with previous studies (Edelmann et al., 2022). No significant gender differences were observed in enjoyment of food, aligning with Wasif et al. (2020). This may be due to shared academic stress and similar exposure to health education, which could reduce gender-based distinctions in attitudes toward food. While emotional under-eating did not show a significant gender difference in our study, previous findings (Sze et al., 2021; Smith et al., 2020) reported higher levels among females. This discrepancy may reflect the influence of campus culture, stress, and dietary knowledge. Significant correlations were found between social media addiction and body dissatisfaction ($r = 0.332$), emotional under-eating ($r = 0.358$), and enjoyment of food ($r = 0.391$). A negative correlation was observed between social media addiction and physical activity ($\rho = -0.204$). These results suggest that higher social media use is linked with greater body concerns, emotional and hedonic eating tendencies, and reduced physical activity—findings echoed by studies such as Erbaş & Gümüş (2020) and Ayyıldız & Şahin (2022).

Importantly, body dissatisfaction, physical activity, and enjoyment of food significantly predicted social media addiction. Body dissatisfaction was the strongest predictor, aligning with research showing how social media fosters idealized body standards and appearance anxiety (Sabancı et al., 2021). Physical activity also emerged as a negative predictor, consistent with literature showing that increased screen time displaces physical

movement (Leggett-James & Laursen, 2022). Enjoyment of food, linked to increased appetite and cravings during social media use (Aslam et al., 2021), further predicted social media addiction. Emotional under-eating, however, was not a significant predictor.

These findings reflect the cultural and academic context of UiTM Puncak Alam, where high academic demands and social norms may drive similar behaviors across genders. Understanding these dynamics is essential for designing interventions. Digital literacy programs, physical activity campaigns, and mental health support can help reduce the negative impacts of social media addiction and foster healthier coping strategies.

CONCLUSION

This study provides valuable insights into the impact of social media addiction on undergraduate Health Sciences students at UiTM Puncak Alam. Female students reported higher levels of social media addiction and body dissatisfaction, while male students were more physically active. Social media addiction was positively associated with body dissatisfaction, enjoyment of food, and emotional under-eating, and negatively associated with physical activity. Body dissatisfaction, physical activity, and enjoyment of food were significant predictors of social media addiction. These findings highlight the need for targeted interventions, including digital literacy programs, promotion of physical activity, and accessible mental health support. A holistic, campus-wide approach can help reduce the negative effects of social media and enhance student well-being.

This study is limited by its cross-sectional design and reliance on self-reported data, which may cause bias. Future research should use longitudinal methods, include a broader student sample, and examine other factors like self-esteem, peer pressure, and academic performance. Targeted interventions may help improve social media use and support student well-being.

ACKNOWLEDGEMENT

The author would also like to thank Dr. Siti Nor Ismalina Isa, a lecturer in biostatistics, from Faculty of Health Sciences, for her assistance with this survey and express our gratitude to all the students who participating in this research. The authors received no financial support for the research.

REFERENCES

- Ahmed, O., Siddiqua, S. J. N., Alam, N., & Griffiths, M. D. (2021). The mediating role of problematic social media use in the relationship between social avoidance/distress and self-esteem. *Technology in Society*, 64, 101485. <https://doi.org/10.1016/j.techsoc.2020.101485>
- Alfaya, M. A., Abdullah, N. S., Alshahrani, N. Z., Alqahtani, A. a. A., Algethami, M. R., Qahtani, A. S. Y. A., Aljunaid, M. A., & Alharbi, F. T. G. (2023). Prevalence and Determinants of Social Media Addiction among Medical Students in a Selected University in Saudi Arabia: A Cross-Sectional Study. *Healthcare*, 11(10), 1370. <https://doi.org/10.3390/healthcare11101370>
- Amor, T. B. (2024). An Investigation of The Correlation Between “Unhealthy Use” Of social media And the Dissatisfaction of Self-Esteem in Young Adults: Findings from A Cross-Sectional Study Conducted in Tunisia. *Deleted Journal*, 1(1), 39–51. <https://doi.org/10.33422/fshconf.v1i1.120>
- Aslam .M, Khan J. G, Khan G., Asif H., Atta N., Rafique A., Tahir T., Ahmed N. & Ayesha. Impact of social media and Peer Pressure on Eating Behaviors of Adolescents Pakistan *Biomedical Journal*,4(2).<https://doi.org/10.54393/pbmj.v4i2.147>
- Aslan, İ., & Yaşar, M. E. (2020). Measuring Social Media Addiction among University Students. *International Journal of Contemporary Economics and Administrative Sciences*,10(2), 468–492. <https://doi.org/10.5281/zenodo.4429749>
- Ayyıldız, F., & Şahin, G. (2022). Effect of social media addiction on eating behaviour, body weight and life dissatisfaction during pandemic period. *British Food Journal*, 124(9), 2980–2992. <https://doi.org/10.1108/bfj-01-2022-0018>
- Cheng, C., Lau, Y., Chan, L., & Luk, J. W. (2021). Prevalence of social media addiction across 32 nations: Meta-analysis with subgroup analysis of classification schemes and cultural values. *Addictive Behaviors*, 117, 106845. <https://doi.org/10.1016/j.addbeh.2021.106845>
- Digennaro, S., & Iannaccone, A. (2023). Check your likes but move your body! How the use of social media is influencing Pre-Teens body and the role of active lifestyles. *Sustainability*, 15(4), 3046. <https://doi.org/10.3390/su15043046>
- Edelmann, D., Pfirrmann, D., Heller, S., Dietz, P., Reichel, J. L., Werner, A. M., Schäfer, M., Tibubos, A. N., Deci, N., Letzel, S., Simon, P., & Kalo, K. (2022). Physical Activity and Sedentary Behaviour in University Students—The role of Gender, age, field of study, Targeted degree, and Study Semester. *Frontiers in Public Health*, 10. <https://doi.org/10.3389/fpubh.2022.821703>
- Erbaş, Ü., & Gümüş, H. (2020). Participation in physical activity and social media addiction in students. *International Journal of Psychology and Educational Studies*, 7(4), 52–60. <https://doi.org/10.17220/ijpes.2020.04.006>
- Griffin, M., Bailey, K. A., & Lopez, K. J. (2022). #BodyPositive? A critical exploration of the body positive movement within physical cultures taking an intersectionality approach. *Frontiers in Sports and Active Living*, 4. <https://doi.org/10.3389/fspor.2022.908580>
- Jiang, W., Luo, J., & Guan, H. (2021). Gender difference in the relationship of physical activity and subjective happiness among Chinese university students. *Frontiers in Psychology*, 12. <https://doi.org/10.3389/fpsyg.2021.800515>
- Kinkel-Ram, S. S., Staples, C., Rancourt, D., & Smith, A. R. (2022). Food for thought: Examining the relationship between low calorie density foods in Instagram feeds and disordered eating symptoms among undergraduate women. *Eating Behaviors*, 47, 101679. <https://doi.org/10.1016/j.eatbeh.2022.101679>
- Krupa-Kotara, K., Grajek, M., Rozmiarek, M., Malchrowicz-Moško, E., Staśkiewicz, W., León-Guereño, P., Aguirre-Betolaza, A. M., & Castañeda-Babarro, A. (2023). The Role of social media in Internalizing Body Knowledge—A Cross- Sectional Study among Women with Different Food Preferences. *International Journal of Environmental Research and Public Health/International Journal of Environmental Research and Public Health*, 20(3), 2069. <https://doi.org/10.3390/ijerph20032069>
- Leggett-James, M. P., & Laursen, B. (2022). The consequences of social media use across the transition into adolescence: body image and physical activity. *Journal of Early Adolescence/the Journal of Early Adolescence*, 43(7), 947–964. <https://doi.org/10.1177/02724316221136043>
- Mahmood, Q. K., Jafree, S. R., & Sohail, M. M. (2020). Pakistani Youth and Social Media Addiction: The Validation of Bergen Facebook Addiction Scale (BFAS).

- International Journal of Mental Health and Addiction, 20(1), 581–594. <https://doi.org/10.1007/s11469-020-00391-0>
- Manap, A. H. A., Ali, H., Devaraj, N. K., Halim, A. N. A., Thaygaraja, T., & Azmi, N. D. N. (2023). Determinants of higher body shape concern among medical students in Universiti Putra Malaysia. *Malaysian Journal of Medicine and Health Sciences/Malaysian Journal of Medicine and Health Sciences*, 19(s17), 16–24. <https://doi.org/10.47836/mjmhs.19.s17.3>
- O'Reily, I., & Richardson, L. (2018). Social media and its associations with body satisfaction, exercise and eating habits on undergraduate students. *Dublin Business School*.
- Pedalino, F., & Camerini, A. (2022). Instagram Use and Body Dissatisfaction: The Mediating Role of Upward Social Comparison with Peers and Influencers among Young Females. *International Journal of Environmental Research and Public Health/International Journal of Environmental Research and Public Health*, 19(3), 1543. <https://doi.org/10.3390/ijerph19031543>
- Sabancı, İ. G., Erensoy, H., & Luş, M. G. (2021a). The relations between social media addiction and negative body perception in Turkish university students. *International Journal of Academic Medicine and Pharmacy/International Journal of Academic Medicine and Pharmacy (Online)*, Volum: 3, Issue: 3(Volum: 3, Issue: 3), 239–244. <https://doi.org/10.29228/jamp.51531>
- Salari N., Zarei H., Far A.H, Mohammadi M, Rasoulpoor S., & Shohaimi S, (2023) The global prevalence of social media addiction among university students: a systematic review and meta-analysis. *Journal of Public Health*, 1-14. <https://doi.org/10.1007/s103989-023-02012-1>
- Smith, J. M., Serier, K. N., Belon, K. E., Sebastian, R. M., & Smith, J. E. (2020). Evaluation of the relationships between dietary restraint, emotional eating, and intuitive eating moderated by sex. *Appetite*, 155, 104817. <https://doi.org/10.1016/j.appet.2020.104817>
- Sze, K. Y. P., Lee, E. K. P., Chan, R. H. W., & Kim, J. H. (2021). Prevalence of negative emotional eating and its associated psychosocial factors among urban Chinese undergraduates in Hong Kong: a cross-sectional study. <https://doi.org/10.1186/s12889-021-10531-3>
- Sze, K. Y. P., Lee, E. K. P., Chan, R. H. W., & Kim, J. H. (2021). Prevalence of negative emotional eating and its associated psychosocial factors among urban Chinese undergraduates in Hong Kong: a cross-sectional study. *BMC Public Health*, 21(1). <https://doi.org/10.1186/s12889-021-10531-3>
- TekiNdur, C., Turğut, M., Özkan, A., & Yağbasan, F. E. (2020). Determination of the relationship between the participation level of university students to physical activity and their social media addiction. *DergiPark (Istanbul University)*. <https://dergipark.org.tr/tr/pub/intjscs/issue/53474/711765>
- Tseng, H., Shanmugam, M., Magalingam, P., Shahbazi, S., & Featherman, M. S. (2022). Managing enterprise social media to develop consumer trust. *British Food Journal*, 124(12), 4626–4643. <https://doi.org/10.1108/bfj-11-2020-0995>
- Wasif, N. S., Sohail, N. M., & Zaheer, N. M. (2020). Eating behaviours as predictors of dissatisfaction with food related life. *Journal of the Pakistan Medical Association*, 1–11. <https://doi.org/10.47391/jpma.751>
- Wang, H., Miao, P., Jia, H., & Lai, K. (2023). The dark side of upward social comparison for social media users: an investigation of fear of missing out and digital hoarding behaviour. *Social Media + Society*, 9(1), 205630512211504. <https://doi.org/10.1177/20563051221150420>
- Xuan, Y. J., & Amat, M. a. C. (2021). The Gender Differences in the Relationships Between Self-Esteem and Life Dissatisfaction with Social Media Addiction Among University Students. *Asian Social Science*, 17(11), 48. <https://doi.org/10.5539/ass.v17n11p48>

Organoleptic Properties Evaluation of Rock Melon (*Cucumis melo*) Qualities on Flesh Sample Post-harvest Day 3

Wan Mazlina Md Saad^{13,*}, Munirah Hannani Rosli¹, Hanis Azmi¹, Nurul Husna Sakinah Mohd Naser¹, Wan Shahrman Yushdie Wan Yusoff¹, Muhammad Fathee Md Bohari².

¹Centre for Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

²Centre of Nutrition and Dietetics, Faculty of Health Sciences, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

³Research and Education in Biomedical, Epidemiology and Immunology Aliances (REBEIA), FSK, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Background: The rock melon, scientifically called *Cucumis melo*, is a fruit characterised by its orange flesh and is often recognised as cantaloupe or melon. The rock melon is highly regarded for its exceptional sweetness and unique aroma, is farmed on a global scale. The cultivation of rock melon has emerged as a highly promising agricultural in Malaysia. However, little to no research has been conducted on the cultivation of rock melon crops in Malaysia, specifically regarding their market potential. This study aims to determine the most appealing quality of rock melon on post-harvest day 3 compared to the control based on organoleptic evaluation. **Methods:** The organoleptic examination of rock melon flesh was performed using a 5-point hedonic scale, comparing control samples to post-harvest day 3 samples. 57 panellists evaluated uniform-sized and equal-weight flesh samples from both groups blindly. Evaluation of the organoleptic comprises the attributes of colour, aroma, firmness, crunchiness, juiciness, sweetness, and overall acceptability. Preference mapping and statistical analysis were used to assess the difference in mean score liking of each attribute. **Results:** In terms of aroma, crunchiness and overall acceptability, the post-harvest day 3 samples showed significant outcomes ($p < 0.05$) compared to the control samples, while the control samples excelled predominantly in colour attributes. **Conclusion:** Preference mapping provided insightful information regarding the qualitative preferences of both samples. The organoleptic responses showed that on post-harvest 3 the best quality of rock melon is on its aroma crunchiness and overall acceptability. This study can empower producers to increase their knowledge and ultimately increase the fruit's marketability.

Keywords:

Cucumis melo; organoleptic; post-harvest; rock melon.

INTRODUCTION

Cucumis melo (C. melo) is part of Cucurbitaceae family that consists of several varieties including muskmelon, rock melon, sweet melon, honeydew, Persian melon, or spanspek (Mariod et al., 2017). Rock melon is widely cultivated in Malaysia and mostly planted in Johor, Kedah, Kelantan Pahang, and Terengganu ((Masde & Mohd, 2016)). Among the locals rock melon known as other common names such as melon or cantaloupe (Khatri et al., 2008; Manchali & Murthy, 2020; Rao & Menon, 2012). There are various ways to eat melon depending on the country, either consumed the fruit raw or made into juice or jam, and the seeds can be used to extract oil (Benmeziane et al., 2018; Manchali et al., 2021). The growing popularity of warmer lifestyles has resulted in a significant increase in the demand for tropical fruit and *Cucumis melo* has gained importance as a commercial crop

Journal homepage: <https://journals.iium.edu.my/ijahs/index.php/IJAHs>
EISSN NO 2600-8491

in the world (Farcuh et al., 2020; Lester, 2006; Masde & Mohd, 2016; Napolitano et al., 2020; Zainal Abidin et al., 2013). The total marketability of rock melon depends on several factors such as climatic conditions, types of soil, harvesting process, and packaging practices used in each growing zone (Lester, 2006). These factors are also lead reason for the wide variances in food quality and customer satisfaction.

A high quality rock melon harvested fully ripe with orange flesh are the most distinct valued for their fruity flavour and high sugar content as consumer preferences focus on its sweetness, colour, juiciness and acceptable aroma and its volatiles compounds. Those attributes resulted from the changes in natural process of physiological and biochemical changes which later gives the orange colour, increasing of the sugar content and decrease in organic acid which later resulting into sweet flavour, fruity aroma and softening of the fruit texture (Beaulieu et al., 2004; Farcuh et al., 2020; Khatri et al., 2008; Lester, 2006;

* Corresponding author.

E-mail address: wanmaz755@uitm.edu.my

Lignou et al., 2014). Melons have a short shelf life once they are fully ripened, therefore consumers find it challenging to determine the quality of these fruits and melons cannot be determined only by their outward look. Therefore, melons are usually harvested semi-ripe to achieve a longer shelf life (Khatri et al., 2008; Lignou et al., 2014).

In a consumer survey of different melon varieties, the overall acceptance strongly correlates with the attributed taste, sweetness and texture (Farcuh et al., 2020). Type of storage is one of the key factors that can affect the sensory characteristics, particularly crunchiness and firmness, which decrease significantly with storage (Beaulieu et al., 2004). Organoleptic evaluation, also known as sensory evaluation (Khatri et al., 2008) is based on human perception, and it provides information about a specific property of a product. However, the study on organoleptic properties, for a specific post-harvest day are still lacking especially in Malaysia. A study showed that organoleptic responses showed that watermelon on post-harvest 4 has best quality and most appealing taste compared to post harvest 8 (Mohamad Salin et al., 2022). Therefore using a hedonic test scale, this study aims to evaluate the organoleptic characteristics of rock melon (Stone, 2018) by determining the colour, aroma, firmness, crunchiness, juiciness, and sweetness of rock melon flesh post-harvest day 3 and determine the overall quality of rock melon flesh post-harvest day 3 using organoleptic evaluation. This is mainly due to the results showed in (Mohamad Salin et al., 2022) study indicating good organoleptic outcomes.

Consumer's current trends emphasise more on taste and quality when selecting and consuming food, which indicates how a successful food industry market should be. The food quality must be appealing, appetising, and enticing and this includes the customers' acceptability in terms of the food quality quality attributes of aroma, taste, aftertaste, tactile features, and appearance (Singh-Ackbarali & Maharaj, 2014). The findings of this study will assist rock melon farmers and retailers to increase their knowledge about the fruits and thus to enhance their marketability in this industry. The aim of the study is to determination of the colour, aroma, firmness, crunchiness, juiciness, and sweetness of Cucumis melo flesh post-harvest day 3 using organoleptic evaluation.

MATERIALS AND METHODS

Sample size calculation for organoleptic evaluation

The sample size for this study was calculated using the

Yamane sample size calculation (Kasiulevičius et al., 2006). The target population for this study was students of Bachelor of Medical Laboratory Technology, UiTM Puncak Alam therefore, the sample size was calculated by using the confidence level of 95% with a margin of error of at 5%. Based on the Yamane's formula calculation, 52 students were recommended to participate in the study. Considering the possible 10% nonresponse, the final sample size was 57 participants.

Sample preparation method

A total of five almost uniformly sizes of rock melons (Cucumis melo) were harvested from the local Hamid Rock Melon farm in Selangor, Malaysia. After rock melons has been harvested, they were stored at 25oC 3 days prior to the evaluation day (Shafinaz et al., 2022). As for the control samples, three rock melons were purchased from the local market a day before evaluation session.

Preparation of samples

The preparation of the sample was conducted at the preparation room, Level 4, FSK, 1,5 (UiTM) Puncak Alam. The rock melon samples were washed beforehand, then patted dry using tissue paper. The outer layer of the fruit and the seeds were completely removed. The flesh of the fruits were cut into a small portion of 15g (Shafinaz et al., 2022). Disposable containers were used to serve the sample and each of the samples were given toothpick as picker and to be used as tool to assess the firmness of the fruit.

Organoleptic evaluation

Questionnaires

The evaluation form used was hedonic scale-based with 5 hedonic scale ratings. A total of seven attributes were included in the questionnaires which are, colour, aroma, firmness, crunchiness, juiciness, sweetness, and overall acceptability. The 5 rating hedonic scale value is described as (1 = dislike very much, 2 = dislike slightly, 3 = neither like nor dislike, 4 = like slightly, 5 = like very much) (Zhi et al., 2016). Description of the attributes were described as follows (Lecholocho et al., 2022; Vallone et al., 2013):

1. Colour: Evenness of colour on the sample surface and degree of a dominant colour.
2. Aroma: The intensity of fruity aroma like mixed juice, a fresh fruity aroma (from none to strong).
3. Firmness: The resistant force required to

compress the sample on teeth, prior smashing into pieces in the mouth.

4. **Crunchiness:** The amount of sound generated when chewing the back teeth, ranging from no sound, like a banana, to a lasting crunchy sound.

5. **Juiciness:** The amount of juice released from the sample when bitten by the incisors.

6. **Sweetness:** The taste of sweetness (from non to very strong).

7. **Overall acceptability:** Depends on the liking of the panels.

Procedure of organoleptic evaluation

All panelist were isolated in a separate area from the evaluation room. Samples, a bottle of plain water, pencil, and questionnaires were distributed and served inside each of the testing cubicle respectively. When the panellists entered the evaluation room, they were instructed to pick whichever seat they desired, and the researcher then verbally instructed them on the organoleptic evaluation. Panellist were required to cleanse their palate by drinking plain water. All participants started the evaluation by tasting the sample and ticking the questionnaires provided to them, according to their preferences. Participants were asked to clean their palate before trying another sample. They were also allowed to taste the sample more than one bite.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 28.0 and Microsoft Office Excel 2019 were used for data entering and data analysis. Prior to data analysis, normality testing and outliers were observed using histogram and box plot respectively. Mean and standard deviation were used to describe numerical data. Using the mean data, a spider-web chart was plotted and independent t-test was used to find significant difference between each attribute. If the *p-value* is less than 0.05 ($p < 0.05$), the difference in scores is considered significant.

RESULTS

Mean score comparison of organoleptic evaluation on rock melon flesh

The spider-web chart indicates the mean score comparison of organoleptic evaluation of rock melon

flesh between post-harvest day 3 and the control sample. The axes are characterised by seven attributes that have been studied in the organoleptic evaluation, and were plotted according to the mean score. Figure 1 shows that in terms of colour, the control sample has a slightly higher average

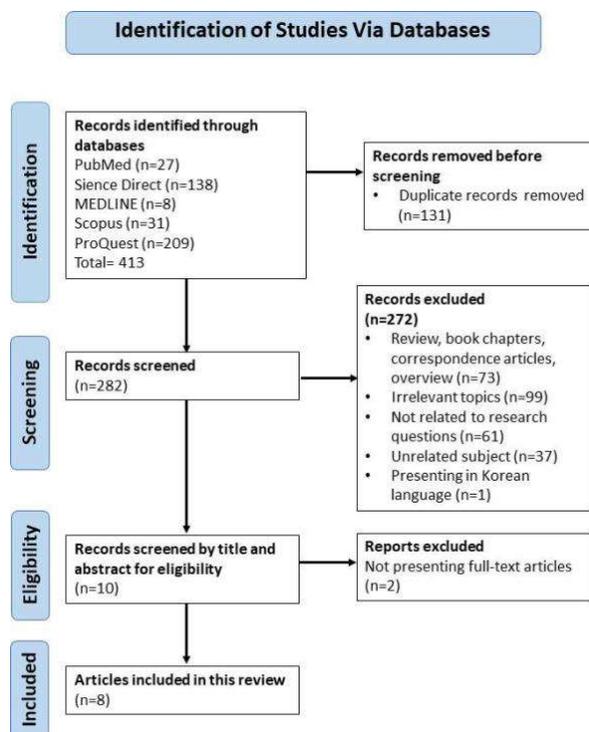


Figure 1: PRISMA flow diagram

value than the post-harvest day 3 sample. In fruit aroma, a significant large difference can be observed visually between the two samples, with post-harvest day 3 sample excelling in this category. Another characteristic that can be observed visually is the crunchiness with overall acceptability of both samples, which may indicate there is slightly big mean difference between the two samples. In terms of firmness, juiciness, and sweetness, both samples are almost equal indicating mean differences between all the three attributes are small.

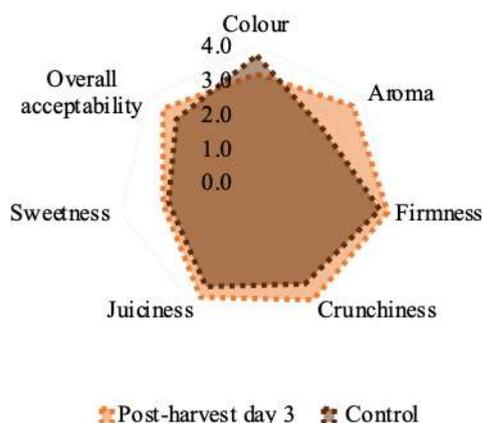


Figure 1: Spider-web chart for organoleptic evaluation of rock melon flesh post-harvest day 3 sample and control sample

Statistical analysis of organoleptic evaluation on rock melon flesh

Statistical analysis for colour attribute

The spider-web chart indicates the mean score comparison of organoleptic evaluation of rock melon flesh between post-harvest day 3 and the control sample. The axes are characterised by seven attributes that have been studied in the organoleptic evaluation, and were plotted according to the mean score. Figure 1 shows that in terms of colour, the control sample has a slightly higher average value than the post-harvest day 3 sample. In fruit aroma, a significant large difference can be observed visually between the two samples, with post-harvest day 3 sample excelling in this category. Another characteristic that can be observed visually is the crunchiness with overall acceptability of both samples, which may indicate there is slightly big mean difference between the two samples. In terms of firmness, juiciness, and sweetness, both samples are almost equal indicating mean differences between all the three attributes are small.

Table 1: Mean scores comparison for colour attribute (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Colour	3.19 (0.79)	3.72(0.98)	-0.53 (-0.86, -0.20)	-3.16 (112)	0.002

Firstly, the independent t-test was conducted to compare the mean score of the post-harvest day 3 sample and the control sample on colour attributes. In the post-harvest day 3 sample, the mean for the colour score was 3.19 + 0.79 (n= 57), and in the control sample, the mean for the colour score was 3.72 + 0.98 (n= 57). The independent t-test revealed a significant difference between the mean score of the post-harvest day 3 sample and control sample (p=0.002). The control sample showed a significantly higher mean score of colour attributes than post-harvest day 3 sample.

Analysis for aroma attributes

The independent t-test for aroma attribute revealed a significant difference between the mean score of post-harvest day 3 sample and control sample (p=<0.001). The post-harvest day 3 sample showed a significantly higher mean score of aroma attributes than the control sample.

Table 2: Mean scores comparison for aroma attribute (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Aroma	3.56 (1.07)	2.49 (1.04)	1.07 (0.68,1.46)	5.42 (112)	<0.001

Analysis for firmness

Table 3: Mean scores comparison for firmness attribute (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Firmness	3.95 (0.88)	3.70 (0.84)	0.25 (-0.07, 0.56)	1.53 (112)	0.13

Third, the mean score of firmness for post-harvest day 3 sample was 3.95 + 0.88 (n= 57) meanwhile the mean score of firmness for control sample was 3.70 + 0.84 (n= 57). The independent t-test showed there was no significant difference in the mean score in firmness between post-harvest day 3 sample and control sample (p= 0.13).

Analysis for crunchiness attributes

Table 4: Mean scores comparison for crunchiness attribute (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Crunchiness	3.75 (1.06)	3.26 (1.13)	0.49 (0.09, 0.90)	2.40 (112)	0.018

On the other hand, in terms of crunchiness, the independent t-test revealed a significant difference between the mean score of post-harvest day 3 sample and control sample (p=0.018).

Analysis for juiciness

Table 5: Mean scores comparison for juiciness attribute (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Juiciness	3.72	3.40 (1.05)	0.31 (-0.06, 0.70)	1.65 (112)	0.102

Likewise, the difference in the mean score for juiciness attribute between the post-harvest day 3 sample and control samples was analysed. The independent t-test findings revealed that there is no significant difference in the mean score between post-harvest day 3 sample and control sample (p= 0.102).

Analysis for sweetness attributes

Table 6: Mean scores comparison for sweetness attribute (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Sweetness	2.82 (1.17)	2.56 (0.95)	0.26 (-0.13, 0.66)	1.323 (112)	0.189

In the same way, the independent t-test findings for sweetness have also revealed that the mean scores between post-harvest day 3 sample and control sample have no significant difference (p= 0.189).

Analysis for overall acceptability

Table 7: Mean scores comparison for overall acceptability (n=57)

Attribute	Post-harvest day 3 (n=57) Mean (SD)	Control (n=57) Mean (SD)	Mean diff (95% CI)	t-stats (df)	p-value
Overall acceptability	3.49 (0.95)	3.00 (1.00)	0.49 (0.13, 0.85)	2.69 (112)	0.008

Lastly, the mean score for the overall acceptability recorded was 3.49 + 0.95 (n= 57) for post-harvest day 3 sample, and in the control sample, the mean for the colour score was 3.00 + 1.00 (n= 57). The independent t-test results indicate there was a significant difference in the mean score in overall acceptability between post-harvest day 3 sample and control samples (p= 0.008).

DISCUSSION

Rock melon contains an abundance amount of beta-carotene, and influenced the fruit orange flesh colour and its characteristic (Nagashima et al., 2021; Park et al., 2018). The statistical analysis is based on the organoleptic evaluation of colour attribute between post-harvest day 3 and control sample. Results show that there is a significant difference, with control sample in colour attribute compared to post-harvest day 3 sample, with mean difference of 0.55. The different colour of the mesocarp itself may be responsible for the variation in colour preferences between the post-harvest day 3 sample and the control sample. Based on this study, control sample that are currently at the market contributed to a better colour quality to consumers compared to the post -harvest day 3 samples.

The aroma is one of the most distinct features of the rock melon with the aroma being used in the industry to get a better flavour of snacks and desserts ((Kermiche et al., 2018). The aroma patterns of fruit have been found differ during different ripening stages and among different cultivars (Fallik et al., 2001; Lamikanra et al., 2003). As mentioned (Lamikanra et al., 2003) the extended post-harvest shelf-life is likely to produce a less volatile aroma,

which subsequently reduce the quality of melon flavour.

Firmness, crunchiness, and juiciness are the three textural attributes that have been studied. The degree to which the fruit softens after harvest is a significant factor in determining its quality and post-harvest longevity (Pech et al., 2008). According to findings, (Faruh et al., 2020; Pouyesh et al., 2017), melon's firmness can vary depending on the genotype of the fruit. In this study, however, no significant difference ($p > 0.05$) has been observed in terms of firmness of post-harvest day 3 and control samples. Therefore, according to the outcomes showed that in terms of firmness of the fruit, the post-harvest day 3 sample and control are both comparable and suitable to be marketed. When consuming food, especially fruits and vegetables, one of the attributes that is sought is to have a crunchy and crispy texture. This is because crunchiness and crispiness are typically associated with the freshness of the product (Fillion & Kilcast, 2002). In this study, a significant difference ($p < 0.05$) between the mean crunchiness scores of the post-harvest day 3 sample and the control sample was discovered, with a mean difference of 0.49. The study's findings indicate that post-harvest day 3 exhibits superior quality in terms of crunchiness and is more suitable for consumption at this stage of harvest, as compared to the control sample.

The organoleptic evaluation revealed that the post-harvest day 3 sample exhibited a mean value higher than the control sample in sweetness. However, no significant difference ($p > 0.05$) were observed between the two samples. In the same study, (Manchali et al., 2021) also claimed that titratable acidity and pH can be markers of fruit pulp acidity, which is known to influence fruit taste. During the ripening process, starch is usually converted into sugar, resulting in decrease in starch content and an increase in sugar content. The soluble solids content (SSC) of fruit juice, assesses the presence of sugars, organic acids, and other soluble elements. A refractometer can be used to acquire this measurement, which provides the percentage of pure sucrose, given as °Brix (Kumar, 2019). Being in an early ripening stage, the post-harvest day 3 sample is no less valuable in terms of sweetness compared to control sample, which is currently on the market. Hence, the post-harvest day 3 rock melon is suitable for consumption and can be sold on the market because its level of sweetness is not significantly different from that of the existing market.

Participants distinctly preferred the overall quality of rock melon from the post-harvest day 3 sample compared to the control sample, which was obtained from the local market. The aroma, firmness, crunchiness, juiciness,

sweetness, and overall acceptability of a rock melon sample post-harvest day 3 were all rated high. However, these differences were not always statistically significant. There is a statistically significant difference ($p < 0.05$) between the post-harvest day 3 sample and the control sample, for overall quality preference between post-harvest day 3 sample having a higher mean score compared to the control sample by a margin of 0.44. (Fallik et al., 2001) have mentioned that the interaction of sugars, organic acid, minerals, and aroma volatile compounds is responsible for the flavour of fresh produce, and as a result, preferences for flavour, sweetness, and texture associated highly with total fruit preference. In-line with the study findings, the post-harvest day 3 rock melon is popular and highly favoured by the panellists compared to the control sample.

CONCLUSION

Ensuring the success of the rock melon on the market requires several considerations, including harvesting period, storage condition, and shipment method, all of which must ultimately satisfy consumer preferences. An organoleptic evaluation was conducted to determine the quality of a specific post-harvest period of rock melon and compare it to the current local market. As determined by the panellists, rock melons harvested explicitly on day 3 are well-liked, as shown by the study, specifically regarding aroma; the post-harvest sample on day 3 scored significantly higher than the control sample. To summarise, post-harvest day 3 exhibited a higher overall preference quality than the control sample.

ACKNOWLEDGEMENT

The authors are especially appreciative to the Hamid Rock Melon farm for helping the research. In addition, the authors would like to express their gratitude to and Centre of Nutrition, Faculty of Health Sciences, UiTM Puncak Alam for enabling the research to be conducted and for offering comprehensive services, resources, and conducive work environment. Their sincere thanks are forwarded to Jimma Agricultural Research Center, the coffee panel, Mr. Nigussie Mekonen, Mr. Kelifa Nesiro, Mr. Abrar Sahile, Mr. Jafar Dawid, and Miss. Seyda Abdu and who were actively involved in sample preparation and cup tasting as a team.

REFERENCES

Beaulieu, J. C., Ingram, D. A., Lea, J. M., & Bett-Garber, K. L. (2004). Effect of harvest maturity on the sensory characteristics of fresh-cut cantaloupe. *Journal of*

- Food Science, 69(7), 250–258. <https://doi.org/10.1111/j.1365-2621.2004.tb13624.x>
- Benmeziane, A., Boulekbache-Makhlouf, L., Mapelli-Brahm, P., Khaled Khodja, N., Remini, H., Madani, K., & Meléndez-Martínez, A. J. (2018). Extraction of carotenoids from cantaloupe waste and determination of its mineral composition. *Food Research International*, 111(May), 391–398. <https://doi.org/10.1016/j.foodres.2018.05.044>
- Farcuh, M., Copes, B., Le-Navenec, G., Marroquin, J., Jaunet, T., Chi-Ham, C., Cantu, D., Bradford, K. J., & Van Deynze, A. (2020). Texture diversity in melon (*Cucumis melo* L.): Sensory and physical assessments. *Postharvest Biology and Technology*, 159. <https://doi.org/10.1016/j.postharvbio.2019.111024>
- Kasiulevičius, V., Šapoka, V., & Filipavičiūtė, R. (2006). Sample size calculation in epidemiological studies. *Gerontologija*, 7(4), 225–231. <https://doi.org/10.13165/AIM.0010>
- Khatri, Y., Wei, S., & Wei, F. (2008). Consumer preferences for rockmelons in Australia. *International Journal of Consumer Studies*, 32(2), 179–183. <https://doi.org/10.1111/j.1470-6431.2007.00649.x>
- Lecholocho, N., Shoko, T., Manhivi, V. E., Maboko, M. M., Akinola, S. A., & Sivakumar, D. (2022). Influence of different rootstocks on quality and volatile constituents of cantaloupe and honeydew melons (*Cucumis melo* L.) grown in high tunnels. *Food Chemistry*, 393(December 2021), 133388. <https://doi.org/10.1016/j.foodchem.2022.133388>
- Lester, G. (2006). Consumer preference quality attributes of melon fruits. *Acta Horticulturae*, 712 I, 175–181. <https://doi.org/10.17660/actahortic.2006.712.17>
- Lignou, S., Parker, J. K., Baxter, C., & Mottram, D. S. (2014). Sensory and instrumental analysis of medium and long shelf-life Charentais cantaloupe melons (*Cucumis melo* L.) harvested at different maturities. *Food Chemistry*, 148, 218–229. <https://doi.org/10.1016/j.foodchem.2013.10.045>
- Manchali, S., & Murthy, K. N. C. (2020). Muskmelon. In A. K. Jaiswal (Ed.), *Nutritional Composition and Antioxidant Properties of Fruits and Vegetables* (pp. 533–546). Elsevier Inc. <https://doi.org/10.1016/b978-0-12-812780-3.00033-7>
- Manchali, S., Murthy, K. N. C., Vishnuvardana, & Patil, B. S. (2021). Nutritional composition and health benefits of various botanical types of melon (*Cucumis melo* L.). *Plants*, 10(9), 1–21. <https://doi.org/10.3390/plants10091755>
- Mariod, A. A., Saeed Mirghani, M. E., & Hussein, I. (2017). *Cucumis melo* var. Cantalupo Cantaloupe. *Unconventional Oilseeds and Oil Sources*, 107–111. <https://doi.org/10.1016/b978-0-12-809435-8.00019-6>
- Masde, R. M. M., & Mohd, N. R. N. (2016). Overview of Melon Industry in Malaysia. *Malaysian Agriculture Research and Development Institute, MARDI*, 2015, 1–6.
- Mohamad Salin, N. S., Aziz, M. A., Sayadi @ Saidi, N. S., Abdul Razak, H. R., & Md Saad, W. M. (2022). Organoleptic Evaluation and Determination of Watermelon (*Citrullus lanatus*) Quality Based on Flesh Samples Between Post-Harvest Day 4 and Post-Harvest Day 8. *ASM Science Journal*, 17, 1–5. <https://doi.org/10.32802/asmsci.2022.863>
- Napolitano, M., Terzaroli, N., Kashyap, S., Russi, L., Jones-Evans, E., & Albertini, E. (2020). Exploring Heterosis in Melon (*Cucumis melo* L.). *Plants*, 9(2), 282. <https://doi.org/10.3390/PLANTS9020282>
- Rao, R., & Menon, S. V. (2012). Nutritional quality of muskmelon fruit as revealed by its biochemical properties during different rates of ripening. *International Food Research Journal*, 19(4), 1621–1628.
- Shafinaz, N., Salin, M., Aziz, M. A., Shafiq, N., Saidi, S., Rashmizal, H., & Razak, A. (2022). Organoleptic Evaluation and Determination of Watermelon (*Citrullus lanatus*) Quality Based on Flesh Samples Between Post-Harvest Day 4 and Post-Harvest Day 8.
- Singh-Ackbarali, D., & Maharaj, R. (2014). Sensory Evaluation as a Tool in Determining Acceptability of Innovative Products Developed by Undergraduate Students in Food Science and Technology at The University of Trinidad and Tobago. *Journal of Curriculum and Teaching*, 3(1), 9–27. <https://doi.org/10.5430/jct.v3n1p10>

- Stone, H. (2018). Example food: What are its sensory properties and why is that important? *Npj Science of Food*, 2(1), 2017–2019. <https://doi.org/10.1038/s41538-018-0019-3>
- Vallone, S., Sivertsen, H., Anthon, G. E., Barrett, D. M., Mitcham, E. J., Ebeler, S. E., & Zakharov, F. (2013). An integrated approach for flavour quality evaluation in muskmelon (*Cucumis melo* L. *reticulatus* group) during ripening. *Food Chemistry*, 139(1–4), 171–183. <https://doi.org/10.1016/j.foodchem.2012.12.042>
- Zainal Abidin, M., Shamsudin, R., Othman, Z., & Abdul Rahman, R. (2013). Effect of postharvest storage of whole fruit on physico-chemical and microbial changes of fresh-cut cantaloupe (*Cucumis melo* L. *Reticulatus* cv. *Glamour*). *International Food Research Journal*, 20(2), 953–960.
- Zhi, R., Zhao, L., & Shi, J. (2016). Improving the sensory quality of flavored liquid milk by engaging sensory analysis and consumer preference. *Journal of Dairy Science*, 99(7), 5305–5317. <https://doi.org/10.3168/jds.2015-10612>

Impact of Gamification in Enhancing the Understanding of Papillary Thyroid Carcinoma Among Medical Laboratory Technology Final Year Students

Wan Shahrman Yushdie Wan Yusoff*, Fatin Nur Syazwani Alias, Ainur Izzati Shahrudin, Muhammad Naqiyuddin Izhar, Mohd Nazri Abu, Wan Mazlina Md Saad

Centre for Medical Laboratory Technology, Faculty of Health Sciences, Universiti Teknologi MARA, UiTM Puncak Alam Campus, 42300 Puncak Alam, Selangor, Malaysia

ABSTRACT

Currently, the most prevalent malignant thyroid tumour is Papillary Thyroid Carcinoma (PTC). Therefore, providing Medical Lab Technology (MLT) students with comprehensive knowledge of PTC should help maintain and improve the accuracy for identifying the PTC. However, students feel it is very challenging to understand PTC, especially recognize the characteristics of cell morphology due to rare samples, tend to forget the knowledge, short class and lab sessions, which results in incorrect diagnosis. Thus, this study aims to explore how CytoUniverse, a physical-based game for learning cytology improves the understanding of PTC among MLT final year students, enabling them to accurately identify it in fine-needle aspiration cytology (FNAC). The study involved 60 MLT students who were randomly assigned to three groups. Group 1 (G1) participants engaged with an interactive physical-based game, which had three components: Tangram Puzzle, Flashcards and Snakes & Ladders. Group 2 (G2) participants received the exact information through a video lecture. Group 3 (G3) received both learning methods. Participants were assessed before the intervention (T1) and after the intervention (T2) to determine the effectiveness of each learning methods. Data were collected, tabulated, and analysed using IBM SPSS version 28. Descriptive analysis assessed the normality of the data while Fisher's exact test, paired t-test, and one-way ANOVA test were used for statistical comparisons. The study results showed significant increase ($P < 0.001$) in mean total knowledge scores from T1 to T2 for all groups. There were also significant differences in mean total knowledge scores between G1 and G2 ($P = 0.012$) at T2. In conclusion, study highlights the efficacy of CytoUniverse, specifically through the physical-based game, in enhancing MLT final-year students' understanding of PTC for accurate identification in FNAC.

Keywords:

papillary thyroid carcinoma; cytology; medical laboratory technology; gamification, physical-based game.

INTRODUCTION

Malignant cells can grow in the tissues of the thyroid gland and cause thyroid cancer ("Thyroid Cancer Treatment (PDQ®)–Patient Version", 2023). The thyroid gland is located near the trachea at the base of the larynx. It has a butterfly-shaped structure comprising a right lobe and a left lobe. The two lobes are connected by a thin tissue called the isthmus. The normal dimensions of the thyroid lobes are around 1.5 cm in thickness and width, with a height of approximately 5 cm (Delgado, 2022).

Thyroid cancer represents approximately 3-4% of all cancer cases and is among the most prevalent types of endocrine tumours (Fallahi et al., 2020). With over 298,000

new cases reported globally in 2012, thyroid cancer stands as the most prevalent form of endocrine malignancy (Aarestrup et al., 2019). The risk of developing thyroid cancer is three times higher in women compared to men. Typically, women are diagnosed in their 40s or 50s, while men tend to be diagnosed in their 60s or 70s. Among all new cases reported in Malaysia in 2020, thyroid cancer accounted for 1.6% of the total incidence ("Thyroid Cancer: Symptoms, Causes, Diagnosis, Treatment | Gleneagles Hospitals", n.d.). According to Director General of Health Malaysia (2020) thyroid carcinoma is the eighth most prevalent cancer among Malaysian women in 2016.

There are four types of thyroid carcinoma. Up to 80% of thyroid cancers are Papillary Thyroid Carcinoma (PTC), while 15% of thyroid cancers are Follicular Thyroid Carcinoma (FTC) (Gawin et al., 2017). Additionally, Anaplastic Thyroid Carcinoma (ATC), the most aggressive

Corresponding author.

E-mail address: wshahrman@uitm.edu.my

Journal homepage: <https://journals.iium.edu.my/ijahs/index.php/IJAHs>

EISSN NO 2600-8491

thyroid cancer, accounts for 1-2% of all thyroid cancer cases. Medullary Thyroid Carcinoma (MTC) comprises 3–5% of thyroid cancers. The 10-year Overall Survival (OS) rates for PTC, FTC, or MTC are 98%, 92%, or 80%, respectively, but the median survival for ATC patients is roughly 6 months (Fallahi et al., 2020). ATC can lead to 15–40 % of thyroid cancer death. Fallahi et al. also states that Differentiated Thyroid Cancer (DTC), which develops from follicular cells, accounts for more than 90% of all thyroid cancer cases. PTC and FTC are both DTC. While for MTC, Parafollicular C cells of the thyroid are where it originates. Undifferentiated or ATC is developing from epithelial cells (Gawin et al., 2017).

The best methods for differentiating between benign and malignant thyroid nodules are neck ultrasonography (US) and Fine Needle Aspiration Cytology (FNAC) (Fallahi et al., 2020). Early detection of smaller tumors is made possible by the widespread use of these techniques. The FNAC test is widely used and considered as a reliable, simple, cost-effective and safe way to diagnose thyroid cancer (Chen et al., 2023; Abdullahi et al., 2022). Individuals with thyroid swellings, FNAC may help them avoid invasive and potentially unneeded surgery. However, Abdullahi et al. explain that FNAC accuracy, sensitivity, specificity, negative predictive value and positive predictive value are dependent on the cytopathologists' and interventional radiologists' prior cytology specimen-related experience. The Bethesda system, which classifies cytology data into six categories, is the framework used to report the results of FNAC (Chen et al., 2023). The six categories are (I) Non-diagnostic, (II) Benign, (III) AUS/FLUS, (IV) Follicular neoplasm, (V) Suspicious for malignancy and (VI) Malignant (Abdullahi et al., 2022).

According to Aguado-Linares & Sendra-Portero (2023), the computer programmer Nick Pelling first used the term "gamification" in 2002. He characterized it as the usage of game designs and aspects in non-gaming domains, such as user interfaces, market research, commercial profits, and education. The incorporation of game design features into the learning context is known as "gamification of learning" (Deterding et al., 2011). Because it offers a captivating teaching style and operates in a setting with no stress, gamification encourages students to learn while having fun (Murillo-Zamorano et al., 2023). According to research made by Rosa-Castillo et al. in 2022, students prefer learning with gamification instead of without it because it fosters engagement, offers feedback, supports goal setting, and improves academic achievement.

Numerous previous research has demonstrated gamification's efficiency in improving student comprehension in various study fields. For example, gamification studies were carried out with 27 students enrolled in the Master of Software Engineering for the Web program. They compare the outcomes of two groups: a control group and one that employed gamification to improve its academic performance. It took two weeks to complete this technique, with five hours of teaching each week. The students believed that gamification was useful for introducing the subject's fundamental concept (Aldalur & Perez, 2023). Other than that, Sánchez- Martín et al. (2017) gamified the entire semester of the primary education degree's topic Matter and Energy. The 36 students were divided into two groups: a control group and the other group that applied gamification. There is no doubt that students who participated more in the game achieved superior academic outcomes.

Therefore, the purpose of this study is to evaluate the efficacy of gamification by enhancing the knowledge of MLT final year students to recognize PTC in FNAC using an interactive physical-based game called CytoUniverse. CytoUniverse comprises of several physically based games that are categorized into three different game types: tangram puzzle, flashcards and snakes & ladders. This gamification technique is employed since it has been proven to be effective in increasing students' involvement in the learning process. Recent research has shown that the application of gamification in education, particularly in the health sciences, is beneficial. According to data gathered from various studies, no intervention research studies using gamification in cytology learning have been conducted.

MATERIALS AND METHODS

Study Design

This study applied randomized control trial (RCT) design with 60 participants, all the MLT final year. The students were randomly assigned to three groups which is Group 1 (G1), Group 2 (G2), and Group 3 (G3). The groups were different concerning the interventions that were conducted on them. G1 played a physical-based game known as, CytoUniverse, comprised of Tangram Puzzle, Flashcards, and Snakes & Ladders games related to PTC. G2 also received the similar information and knowledge through a video lecture mode. The physical-based game was delivered to G3 together with the video lecture. A pre-test was taken of the participants before the implementation of the intervention (T1) while a post-test was taken immediately after the intervention (T2).

Study participants

The study was carried out among the MLT final year students of UiTM only and called for the selection of 60 students. The inclusion criteria for the study were as follows: (a) UiTM MLT final year students that are studying at UiTM Puncak Alam and (b) students who have learned cytology in the previous semesters. Students who did not meet these conditions were not included in the study.

Study Setting

This study was conducted at UiTM Puncak Alam, Selangor and involved MLT final year students of UiTM in 2024. The study duration was scheduled from April to June.

Research Tools

Physical-based game

Participants in G1 and G3 played a game called CytoUniverse, which is a physical-based game designed for use in this research. This set of game comprises various types of games such as Tangram Puzzle, Flashcards and Snakes & Ladders. Prior to the study, the game went through some pilot testing with groups of three people including the supervisor of this study to determine the feasibility of using the game in the current research [Figure 1]. The Flashcards game consisted of picture questions concerning the anatomy of thyroid gland, risk factors, symptoms, diagnosis, treatment, FNAC, and cell morphology of PTC in thyroid cancer [Figure 2]. The questions were written on one side with the answers written on the other side of the card. Through this game, they improved their abilities to determine PTC cytomorphology as well as gained more knowledge on this disease. It was therefore created in a way that makes it effective in aiding memorization processes.



Figure 1: Pilot testing of the game for feasibility in study

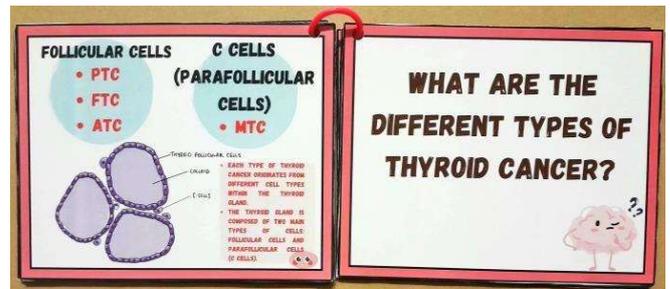


Figure 2: Flashcards

For Tangram Puzzle, participants were provided with 2 sets of cytology images illustrating different types of cell nucleus morphological features [Figure 3]. They got engaged in classifying these cytology images based on certain nuclear morphological features known to be characteristic of PTC. Then, participants were asked to rearrange the Tangram puzzle pieces to match the nucleus structures described in the questions. The answer was given after they fully arranged the puzzle to help them have better understanding about the nuclear morphology of PTC.



Figure 3: Tangram Puzzle

The Snakes & Ladders game was designed to allow players to navigate the board, encountering different challenges and questions related to PTC cytology at certain points [Figure 4]. To initiate their turn, players rolled the dice to determine the number of spaces they would advance on the game board. Certain spaces in the game presented PTC cytology-related questions, which could be in the form of words or pictures. These questions covered microscopic features of normal thyroid cells and PTC cells, including background morphology, nuclear characteristics and cellular arrangements. Correctly answering these questions allowed players to progress on the board or climb up, gaining advantages in the game. However, providing incorrect answers resulted in setbacks or disadvantages

for players. The game benefited participants by providing an enjoyable and dynamic method to enhance their understanding of PTC cytology, promoting active learning and retention of information. The completion of all games typically took approximately 20-25 minutes.



Figure 4: Snakes and Ladders

Video Lecture

The participants in G2 and G3 watched a video lecture titled “Papillary Thyroid Carcinoma” delivered by the researcher in English and in the form of a PowerPoint presentation. The video lecture was published on the YouTube platform and the participants were able to view it through a link provided to them by the researcher through WhatsApp within five days. Before dissemination, the quality of the delivered video lecture was checked through pilot testing with 3 participants, including the supervisor. This testing was done to assess the fact-checking, arrangement, grammar, spelling, syntax and clarity for viewers.

Information in the physical-based game and video lecture

The physical-based game was conducted face to face while the video lectures were delivered online. All groups were provided with same information of PTC in cytology including risk factors, symptoms, diagnosis, and treatment of thyroid cancer, FNAC of thyroid, Bethesda system for reporting thyroid cytopathology, background morphology, cell morphology, and cell arrangement in normal thyroid cells and PTC cells. Thus, this avoided any possibility of one group being given more help than the other, all participants got similar ground information on the topic under discussion.

Questionnaire

Online self-administered survey questionnaires were used to assess participants’ knowledge at two time points: T1 and T2. These questionnaires, distributed through Google Forms, included Questionnaire 1 (Q1) at T1 and Questionnaire 2 (Q2) at T2. In the initial two sections of Q1, participants provided their consent for participation in the study and shared their demographic information (age and

gender). The remaining three sections of Q1 aimed to assess the students’ understanding of PTC in thyroid cancer, specifically focusing on pathophysiology and cytology. These questions were derived from previous studies and were specifically developed for this research. Each question was a multiple-choice question (MCQ), with total scores determining knowledge levels. The questions underwent validation and reliability checks by a lecturer who a cytology expert was to ensure content accuracy and quality.

Table 1: Q1 was distributed to participants before intervention.

Q1	
Section 1	Consent Form
Section 2	Demographics
Section 3	Knowledge on Pathophysiology
Section 4	Background of Cell Cytomorphology
Section 5	Cell Cytomorphology

Q2 consisted of initial sections that captured students’ demographics and group identification after random assignment. The second, third, and fourth sections contained the same questions as Q1 regarding the pathophysiology and cytology of PTC, but the order of the questions was rearranged. The fifth section, administered only to G1 and G3, evaluated participants’ experiences with the physical-based games using a 5-point Likert scale ranging from “strongly disagree” to “strongly agree.” This section included questions adapted from previous research and newly created by the researcher.

Table 2: Q2 was distributed to participants after intervention.

Q2	
Section 1	Demographics and Identification of group
Section 2	Knowledge on Pathophysiology
Section 3	Background of Cell Cytomorphology
Section 4	Cell Cytomorphology
Section 5	Feedback on Game Experience

All questions in Q1 and Q2 were in English. Ensuring content validity involved conducting a pilot study with three individuals, including a lecturer, who tested the questionnaire’s content and structure. This process helped identify potential issues or areas for improvement. Additionally, the structure and content of Q1 and Q2 were altered to prevent bias in pre- and post-intervention results, thus maintaining the integrity of the evaluation process.

Statistical analysis

IBM SPSS version 28 was utilized for data collection, tabulation, and statistical analysis. Descriptive analysis was employed to assess the normality of the data. Numerical data, including knowledge scores and age, were described using mean and standard deviation (SD). While for categorical data, such as gender and group categories, were reported as frequencies and percentages. The study applied Fisher's exact test, paired t-test, and one-way ANOVA test for statistical comparisons.

RESULTS

Participants' Demographic Background

A total of 60 participants completed the questionnaire, with every questionnaire fully answered. After randomization, participants were divided evenly into three groups: G1, G2, and G3, each with 20 participants (33.3%). The response rate was 100%, as all participants completed the questionnaires at both T1 and T2. There were no missing values in the data. Participants' ages ranged from 22 to 26 years, with an average age of 23.65 and a standard deviation of 1.48. Most participants were female, comprising 85% of the sample. Table 3 shows the traits of the participants.

Fisher's exact test was conducted with a significance level (α) of 0.05 to assess the gender distribution among the three groups. The results revealed no statistically significant difference in gender distribution among the groups, χ^2 ($N = 60$), $P = 0.900$. Thus, the gender distribution was similar across all three groups, and no significant differences were observed. This suggests that the randomization process was effective and ensured an equity representation of participants across the study groups.

Table 3. Traits of the participants.

Variables	Category	Group 1 (n = 20)	Group 2 (n = 20)	Group 3 (n = 20)
Gender (n, %)	Male	2 (10.0)	4 (20.0)	3 (15.0)
	Female	18 (90.0)	16 (80.0)	17 (85.0)
Age (mean, SD)		23.70 (1.13)	23.50 (0.83)	23.75 (1.37)

Quantitative Assessment Scores for Level of Knowledge

Table 4 presents the mean total knowledge score, standard deviation (SD), minimum and maximum values for G1, G2, and G3 at both T1 and T2. In the survey assessment at T1, scores ranged from a minimum of 0 to a maximum of 26. While at T2, the scores ranged from a minimum of 17 to a maximum of 32. The paired t-test results shown in Table 5 indicate a significant increase in mean total knowledge scores from T1 to T2 for all groups. For G1, mean total knowledge score increased significantly from T1 to T2 ($t(20) = -13.61$, $P < 0.001$). As a result, the mean of the post-intervention knowledge score (mean = 30.15, SD = 2.76) was significantly higher than the pre-intervention (mean = 11.00, SD = 5.04), with a 95% confidence interval for the mean difference between pre-intervention and post-intervention as -22.10 and -16.21. Similarly, for G2, which increased significantly from T1 to T2 ($t(20) = -8.03$, $P < 0.001$). Thus, the mean of the post-intervention knowledge score (mean = 26.80, SD = 3.71) was significantly higher than the pre-intervention (mean = 12.80, SD = 5.89), with a 95% confidence interval for the mean difference between pre-intervention and post-intervention as -17.65 and -10.35. The mean total knowledge score for G3 also saw a significant increase from T1 to T2 ($t(20) = -9.70$, $P < 0.001$). The mean of the post-intervention knowledge score (mean = 29.20, SD = 3.68) was significantly higher than the pre-intervention (mean = 14.20, SD = 5.36), with a 95% confidence interval for the mean difference between pre-intervention and post-intervention as -18.24 and -11.77. Bar chart in Figure 5 illustrates the changes in mean total knowledge scores for each group from T1 to T2, demonstrating the overall improvement across all groups.

Table 4. The mean total knowledge score of G1, G2, and G3 at T1 and T2.

Variable	Category	Time of intervention	Mean	SD	Min	Max
Group	G1	T1	11.00	5.04	0	20
		T2	30.15	2.76	25	32
	G2	T1	12.80	5.89	5	26
		T2	26.80	3.71	17	32
	G3	T1	14.20	5.36	6	25
		T2	29.20	3.68	18	32

Table 5: Change in the mean of total knowledge scores before (T1) and after (T2) intervention among 60 respondents.

Variable	Group	Pre-intervention mean (SD)	Post-intervention mean (SD)	Mean difference (95% CI)	t-stats (df)	P
Knowledge score	G1	11.00 (5.04)	30.15 (2.76)	-19.15 (-22.10, -16.21)	-13.61 (19)	< 0.001
	G2	12.80 (5.89)	26.80 (3.71)	-14.00 (-17.65, -10.35)	-8.03 (19)	< 0.001
	G3	14.20 (5.36)	29.20 (3.68)	-15.00 (-18.24, -11.77)	-9.70 (19)	< 0.001

The paired t-test results indicate a significant increase in mean total knowledge scores from T1 to T2 for all groups.

Table 6: Comparison of mean knowledge scores for G1, G2 and G3 at T1 and T2.

Variables	G1 (n = 20)	G2 (n = 20)	G3 (n = 20)	F-stats ^a (df)	P		
					G1-G2	G1-G3	G2-G3
T1 (mean, SD)	11.00 (5.04)	12.80 (5.89)	14.20 (5.36)	1.74 (2, 57)	0.581	0.186	0.719
T2 (mean, SD)	30.15 (2.76)	26.80 (3.71)	29.20 (3.68)	5.13 (2, 57)	*0.012	0.680	0.093

^aOne-way ANOVA. The Scheffe test is employed to conduct multiple comparisons to decide if there are statistically significant differences in knowledge scores among all groups. (*) indicate (P < 0.05) which there was a significant difference between G1 and G2 at T2.

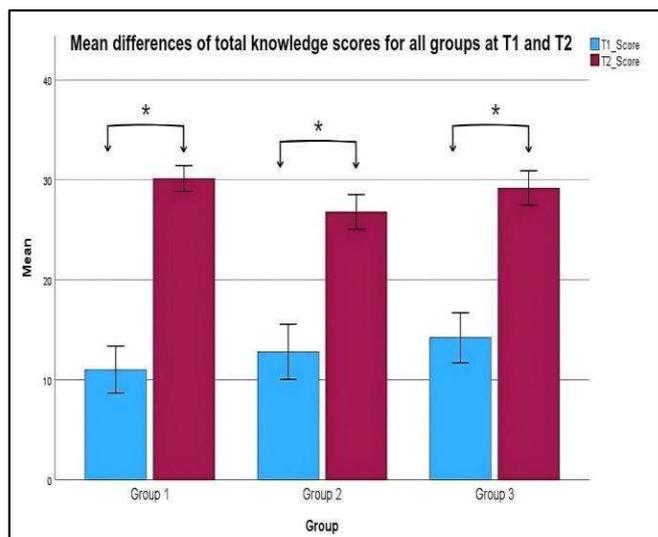


Figure 5: Changes in mean total knowledge scores for each group from T1 to T2. (*) indicates a significant change (P < 0.001) in the mean total knowledge scores for all groups before and after the intervention.

A one-way ANOVA test was used to compare the impact of gamification on knowledge scores among G1, G2, and G3 at both T1 and T2. At T1, there was no significant effect of gamification on knowledge scores across all groups, with P > 0.05 level for all groups (F (2, 57) = 1.74, P = 0.185, η² = 0.058). However, at T2, gamification had a significant effect on knowledge scores between “G1 and G2”, with P < 0.05 (F (2, 57) = 5.13, P = 0.009, η² = 0.152) [Table 6]. Scheffe’s test for multiple comparisons revealed that at T1, there were no significant differences in mean knowledge scores between G1 and G2 (P = 0.581), G1 and G3 (P = 0.186), or G2 and G3 (P = 0.719). At T2, however, there was a significant difference between G1 and G2 (P = 0.012), meanwhile the differences between G1 and G3 (P = 0.680), and G2 and G3 (P = 0.093) were not significant.

Qualitative Survey Results

Table 7 represents questions concerning participants’ experiences and opinions regarding the intervention physical-based game. These questions were specifically included in the questionnaires for participants from G1 and G3 (n = 40) only. Responses were recorded based on a 5-point Likert scale, ranging from 1 (strongly disagree) to 5 (strongly agree). Mean scores falling within 1–1.80 suggest strong disagreement, while scores between 1.81 and 2.60 show disagreement [Table 8]. Scores within the range of 2.61–3.40 specify for neutral. Mean scores ranging from 3.41 to 4.20 imply agreement with the statement, while scores from 4.21 to 5 imply strong agreement. The survey results, detailing participants’ experiences, and opinions regarding the physical-based game as an intervention are summarized in Figure 6.

Table 7: Descriptive statistics on the participants’ participations and perceptions regarding the physical-based game as an intervention.

	n	Min	Max	Mean	SD
Q1-Utilizing gamification is an effective approach to learn the fundamentals of cytology.	40	4	5	4.77	0.42
Q2-Gamification serves as a source of motivation for me to delve deeper into the study of cytomorphology.	40	4	5	4.75	0.44
Q3-Gamification improves my comprehension of the subject being taught.	40	3	5	4.77	0.48
Q4-The feedback system integrated into the gamification during game play sessions enhanced my comprehension of the topic.	40	4	5	4.80	0.41
Q5-The gamification experience strengthened my motivation to learn cytology.	40	4	5	4.80	0.41
Q6-I experienced a sense of satisfaction upon completing the game task.	40	3	5	4.78	0.48
Q7-The time required to complete the game is reasonable.	40	4	5	4.75	0.44
Q8-The physical-based game offers comprehensive and detailed material pertaining to the subject matter.	40	4	5	4.77	0.42
Q9-I highly recommend the game to others.	40	4	5	4.85	0.36

5-Strongly agree, 4-Agree, 3-Neutral, 2-Disagree, 1-Strongly disagree

Table 8: Qualitative interpretation of 5-point Likert scale measurement.

Likert scale description	Likert scale	Interval
Strongly disagree	1	1.00-1.8
Disagree	2	1.81-2.6
Neutral	3	2.61-3.4
Agree	4	3.41-4.2
Strongly agree	5	4.21-5.0

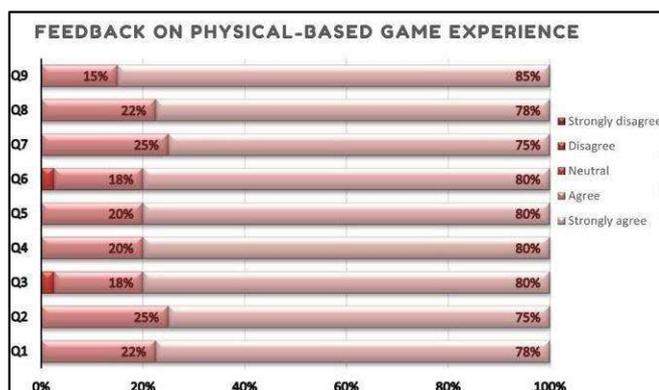


Figure 6: Analysis of participants feedback on physical-based game engagement

DISCUSSION

The study aimed to explore how gamification may improve MLT final year students to have better understanding about PTC, so they are able to identify it accurately in FNAC. Following the implementation of the physical-based game, there was a noticeable improvement in students' knowledge scores for identifying PTC cases. Both G1 and G3 exhibited significantly higher mean total knowledge scores. Although G2 also showed a significantly increase in mean total knowledge scores after watched the conventional video lecture, but the results were lower than G1 and G3.

Before the intervention (T1), the minimum and maximum scores from the Q1 were difference across all groups. Nevertheless, after the intervention (T2), the Q2 evaluation showed that while the maximum score was similar across all groups, the minimum scores varied. Participants in G1 had a minimum score of 25, G2 had 17, whereas those in G3 had 18. Despite these variations, only the differences between G1 and G2 was statistically significant. The modest improvement observed in G2 participants, who received video lectures, indicates that video lectures alone are insufficient to boost students' comprehension of PTC cytology, unlike the physical-based game intervention that benefited G1 and G3 participants.

The lower improvement in G3 compared to G1 suggested that combining two different learning methods might have led to cognitive overload, making it more difficult for participants to process and retain the information effectively. Moreover, the integration of both methods might have decreased the engagement level contrasted with solely playing the physical-based game.

The outcomes of this study agree with previous intervention research by Lampropoulos and Sidiropoulos (2024), Alsswey and Malak (2024), and Le et al. (2024). These studies have also shown that gamification significantly improves knowledge acquisition in different education fields such as cytology, operating system, and traffic knowledge. Regardless of differences in study environments and other aspects, the findings emphasize the main conclusions of this research. Further discussion on these variations and aspects is presented in the following section.

The research by Le et al. (2024) evaluated a gamified e-learning platform's effect on traffic knowledge and skills among Vietnamese adolescents using a quasi-experimental design. The results showed significant improvement in the intervention group's traffic knowledge and skills immediately after the intervention, contrasted the control group. A quasi-experimental study can be biased because it lacks random assignment, leading to differences between the intervention and control groups that are unrelated to the intervention itself. Our research is superior to this study as we used a RCT approach to mitigate potential bias. By randomly assigning participants to one of three different intervention groups, we increased the study's capability to compare the intervention group with a control group, making our results more reliable.

A study was made by Alsswey and Malak in 2024 to examine the effects of the gamified learning tool "Kahoot!" on stress, anxiety, self-efficacy, and academic achievements among university students in Jordan. Using a pre-posttest control group design, 89 in the experimental group (used traditional learning alongside Kahoot!) and 87 in the control group (used only traditional methods). Results showed that the experimental group had reduced stress, anxiety and improved self-efficacy and academic achievements. The study suggests integrating Kahoot! into university teaching can increase students' psychological well-being and academic performance. In contrast to this study, a physical-based game was developed, concentrating primarily on delivering correct information, best visual aids, and matter related to PTC in cytology. This focused approach empowered a more targeted evaluation of participants' knowledge enhancement, leading to more accurate outcome measurements.

In their earlier study, Lampropoulos and Sidiropoulos (2024) made a three-year longitudinal study involving 1001 higher education students compared the impact of gamified learning, traditional methods, and online learning on academic performance for subject of "Operating Systems". Gamified learning consistently defeated both traditional and online methods across success rates, excellence rates, and average grades in both theoretical and laboratory components of the course. The integration of gamification elements such as leaderboards, badges, scores, points, and anonymized nicknames in the course platform and evaluation system played a significant role. Allied to this study, immediate feedback was provided at the conclusion of each question in the game, allowing participants to fix their mistakes and increase their understanding. Ogino and Matsuguma (2024) state that in gamification, the importance of generating enjoyment through immediate feedback cannot be undervalued, as it concedes for learning from past actions and improves the quality of behavior.

The board game such as Snakes & Ladders helped participants learn about PTC in a fun and enjoyable way, improving their focus and attention during the learning process. Its attractive design, gameplay element, rewards, and challenges encouraged participants to keep playing. Another advantage of board games is it boosts players learning and social skills. On top of that, Karbownik et al. (2016) discovered that participation in the board game fosters clinical thinking and peer interactions, encouraging a more contextualized and deeper learning approach. This may translate into better cognitive outcomes over time. According to the findings of this study and previous research, we propose that effective implementation of gamification holds great potential as an educational approach for teaching PTC in cytology. Additionally, the simplest yet innovative design of the physical-based game used in this study allows for easy adaptation to various subjects, facilitating education on a wide range of topics. The game is user-friendly, and the design can be easily modified for other subjects by only changing the questions, making it a flexible tool for education. However, further research is vital to reinforce and validate this affirmation. Additional studies may offer more evidence and insights into the efficacy of gamification across various educational subjects and fields.

In this study, all participants agreeing on the effectiveness of gamification as a learning method for cytology, as it increased their understanding of the subject. The findings also showed that the feedback mechanism integrated into the physical-based game was suitable and aided in the participants' improved comprehension during gameplay. Moreover, all participants (100%) felt that gamification

serves as a source of motivation for them to delve deeper into the study of this topic. These results are consistent with those of the research by Cortés-Pérez et al. (2023), who found that Kahoot! activities motivated over 65% of students to study every week. Consequently, all participants expressed a willingness to recommend the physical-based game to others.

The feedback results also showed unanimous agreement among all participants regarding the optimal timing for completing each game. The duration was considered reasonable, allowing participants to glean maximum information from the physical-based game. Furthermore, the feedback indicated that the content of the developed physical-based game was well-structured, comprehensible, and enriched with pertinent information on the subject.

This study highlighted several significant strengths that bolstered its reliability. Firstly, the use of an RCT design effectively minimized bias and confounding variables, thereby heightening the credibility of the findings. Secondly, the intervention introduced CytoUniverse, a physical-based game that provides hands-on learning, encourages social interaction, and minimizes screen time. They're flexible for different educational needs and work effectively in environments with limited access to technology. The study also maintained a zero-dropout rate due to high student engagement, supported by thorough pre-intervention and post-intervention evaluations tailored to assess the game's impact on understanding PTC accurately. Unlike other game designs, CytoUniverse incorporated a feedback mechanism to help students correct misconceptions and enrich their learning experience. This feature ensured that students received immediate guidance and support, strengthened their understanding and retention of the material in a more comprehensive manner compared to conventional methods.

Nevertheless, it is important to acknowledge several limitations inherent in this study. The small sample size of 60 participants constrains the generalizability of the findings. Subsequent studies should include a more diverse samples from Malaysia's student population to increase validity and reliability. Moreover, using participants with no prior knowledge of PTC is recommended to ensure that any observed improvements are directly attributed to the gamification intervention rather than prior knowledge, enhancing robustness of the results. Furthermore, this research lacks a follow-up mechanism to evaluate the retention of information post-intervention. Therefore, future studies must integrate longer follow-up periods to assess the durability of

information retention. Other than that, the lengthy duration required to complete the Snakes & Ladders game must be addressed to maintain engagement. Reducing the number of squares and snakes on the board, as well as, simplifying the rules can make the game quicker and easier to play. Lastly, one participant scored zero on Q1, possibly due to not taking it seriously, misunderstood its purpose, or rushed through it. Researchers should emphasize the importance of careful and accurate responses to both questionnaires to improve future studies. Clear instructions and explanations about the questionnaires' purposes should help ensure participants understand and provide reliable data.

CONCLUSION

In summary, this study highlights the efficacy of gamification, specifically through the physical-based game called CytoUniverse, in enhancing MLT final-year students' understanding of PTC for accurate identification in FNAC. High satisfaction and motivation levels, along with key gamification elements like immediate feedback and interactive learning, ensure engaging and potent teaching methods. Despite that, for further research it is recommended to widen sample sizes to improve the generalizability of the findings, include individuals with no prior knowledge of PTC, shorten and simplify the Snakes & Ladders game, longer follow-up periods to evaluate information retention, and improve engagement strategies.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Centre of Medical Laboratory Technology Studies, Faculty of Health Sciences, UiTM Puncak Alam for enabling the research to be conducted and for offering comprehensive services, resources, and conducive work environment.

REFERENCES

Aarestrup, J., Kitahara, C. M., & Baker, J. L. (2019). Birthweight and risk of thyroid cancer and its histological types: A large cohort study. *Cancer Epidemiology*, 62, 101564. <https://doi.org/10.1016/j.canep.2019.07.003>

Abdullahi, I., Yasin, N. F., Dirken, E. S., Mohamoud, A. M., Guler, I., & Adani, A. A. (2022). Comparative study of fine needle aspiration cytology and histopathology in thyroid nodules at a tertiary care hospital: First report from Somalia. *Asian Journal of Surgery*. <https://doi.org/10.1016/j.asjsur.2022.11.097>

Aguado-Linares, P., & Sendra-Portero, F. (2023). Gamification: Basic concepts and applications in radiology. *Radiología*, 65(2), 122–132. <https://doi.org/10.1016/j.rxeng.2022.10.014>

Aldalur, I., & Perez, A. (2023). Gamification and discovery learning: Motivating and involving students in the learning process. *Heliyon*, 9(1), e13135. <https://doi.org/10.1016/j.heliyon.2023.e13135>

Allswey, A., & Malak, M. (2024). Effect of using gamification of “Kahoot!” as a learning method on stress symptoms, anxiety symptoms, self-efficacy, and academic achievement among university students. *Learning and Motivation*, 87, 101993. <https://doi.org/10.1016/j.lmot.2024.101993>

Balasubramanian, S. P. (2020). Anatomy of the thyroid, parathyroid, pituitary and adrenal glands. *Surgery (Oxford)*, 38(12), 758–762. <https://doi.org/10.1016/j.mpsur.2020.10.009>

Bassanelli, S., N, V., Bucchiarone, A., & Marconi, A. (2022). Gamification for behavior change: A scientometric review. *Acta Psychologica*, 228, 103657. <https://doi.org/10.1016/j.actpsy.2022.103657>

Bchir, A., Bdioui, A., Zammel, H., Missaoui, N., Hmissa, S., & Mokni, M. (2021). The importance of using fine-needle aspiration cytology in the diagnosis of thyroid nodules. *Annals of Medicine and Surgery*, 63. <https://doi.org/10.1016/j.amsu.2021.01.101>

Borges, F. A., Camilo-Júnior, D. J., De Almeida, E. H. C., Louzada, M. J. Q., & Xavier-Júnior, J. C. C. (2023). Score system for cytological evaluation of fine-needle aspiration specimens to predict the diagnosis of papillary thyroid carcinomas. *Annals of Diagnostic Pathology*, 64, 152129. <https://doi.org/10.1016/j.anndiagpath.2023.152129>

Capdevila, J., Awada, A., Führer, D., Leboulleux, S., & Pauwels, P. (2022). Molecular diagnosis and targeted treatment of advanced follicular cell-derived thyroid cancer in the precision medicine era. *Cancer Treatment Reviews*, 106, 102380. <https://doi.org/10.1016/j.ctrv.2022.102380>

- Chen, D. W., Lang, B. H. H., McLeod, D. S. A., Newbold, K., & Haymart, M. R. (2023). Thyroid cancer. *The Lancet*, 401(10387), 1531–1544. [https://doi.org/10.1016/s0140-6736\(23\)00020-x](https://doi.org/10.1016/s0140-6736(23)00020-x)
- Cortés-Pérez, I., Zagalaz-Anula, N., Del Carmen López-Ruiz, M., Díaz-Fernández, Á., Obrero-Gaitán, E., & Osuna-Pérez, M. C. (2023). Study Based on Gamification of Tests through Kahoot!™ and Reward Game Cards as an Innovative Tool in Physiotherapy Students: A Preliminary Study. *Healthcare*, 11(4), 578. <https://doi.org/10.3390/healthcare11040578>
- Cuellar, A. a. C., Rivera, D., Fierro-Maya, L. F., & Valencia, H. P. (2020). Exposure to radiotherapy for first primary cancer as a risk factor for second primary thyroid cancer: A case-control study. *Annales D Endocrinologie*, 81(6), 539–544. <https://doi.org/10.1016/j.ando.2020.11.005>
- Delgado, P. (2022, December 15). WHAT IS A NORMAL THYROID SIZE? FLUOPTICS. FLUOPTICS. <https://fluoptics.com/en/what-is-a-normal-thyroid-size/#:~:text=The%20thyroid%20is%20a%20gland,nec k%20and%20measure s%205%20cm>
- Deterding, S., Dixon, D. A., Khaled, R., & Nacke, L. E. (2011). From game design elements to gamefulness. <https://doi.org/10.1145/2181037.2181040>
- DG of Health. (2020, January 3). Kenyataan Akhbar KPK 3 Januari 2020 – Penerbitan Malaysia National Cancer Registry Report 2012 – 2016 (MNCRR). From the Desk of the Director-General of Health Malaysia. <https://kpkasihatan.com/2020/01/03/kenyataan-akhbar-kpk-3-januari-2020-penerbitan-malaysia-national-cancer-registry-report-2012-2016-mncrr/>
- Duc, N. M., Lee, Y., Park, J. Y., & Lee, B. (2022). An ensemble deep learning for automatic prediction of papillary thyroid carcinoma using fine needle aspiration cytology. *Expert Systems With Applications*, 188, 115927. <https://doi.org/10.1016/j.eswa.2021.115927>
- Fallahi, P., Ferrari, S., Elia, G., Ragusa, F., Patrizio, A., Paparo, S. R., Marone, G., Galdiero, M. R., Guglielmi, G., Foddìs, R., Cristaudo, A., & Antonelli, A. (2020). Primary cell cultures for the personalized therapy in aggressive thyroid cancer of follicular origin. *Seminars in Cancer Biology*, 79, 203–216. <https://doi.org/10.1016/j.semcancer.2020.06.013>
- Ferrante, M., Cristaldi, A., Okatyeva, V., Lo Bianco, S., Conti, G. O., Zuccarello, P., Copat, C., Caltabiano, R., & Cannizzaro, M. A. (2019). Physical Activity and Thyroid Cancer Risk: A Case-Control Study in Catania (South Italy). *International Journal of Environmental Research and Public Health*, 16(8), 1428. <https://doi.org/10.3390/ijerph16081428>
- Gawin, M., Wojakowska, A., Pietrowska, M., Marczak, Ł., Chekan, M., Jelonek, K., Lange, D., Jaksik, R., Gruca, A., & Widlak, P. (2017). Proteome profiles of different types of thyroid cancers. *Molecular and Cellular Endocrinology*, 472, 68–79. <https://doi.org/10.1016/j.mce.2017.11.020>
- Güney, G., & Şahiner, İ. T. (2017). Malignancy Rates of Thyroid Cytology: Cyst Fluid Benign or Non-Diagnostic? *Medical Science Monitor*, 23, 3556–3561. <https://doi.org/10.12659/msm.905718>
- Han, L., Cao, Q., Xie, T., Chen, X., Liu, Y., & Bai, J. (2021). Exploring the experience of nursing undergraduates in using gamification teaching mode based on the flow theory in nursing research: A qualitative study. *Nurse Education Today*, 107, 105158. <https://doi.org/10.1016/j.nedt.2021.105158>
- He, J., Zhang, C., Hu, M., Wu, H., Lu, X., Hao, J., & Huang, F. (2021). Reproductive and menstrual factors for papillary thyroid cancer risk: A case-control study in Chinese women. *Cancer Epidemiology*, 73, 101964. <https://doi.org/10.1016/j.canep.2021.101964>
- How it's found. (n.d.). Thyroid Cancer Canada. <https://www.thyroidcancercanada.org/en/thyroid-cancer/how-its-found>
- Kakamba, J. B., Sabbah, N., Bayauli, P., Massicard, M., Bidingija, J., Nkodila, A., Mbunga, B. K., Ditu, S., Beckers, A., & Potorac, I. (December 2021). Thyroid cancer in the Democratic Republic of the Congo: Frequency and risk factors. *Annales D'Endocrinologie*, 82(6), 606–612. <https://doi.org/10.1016/j.ando.2021.09.002>
- Kapral, N., & Khot, R. (2022). Thyroid anatomy and ultrasound evaluation. *Techniques in Vascular and Interventional Radiology*, 25(2), 100818. <https://doi.org/10.1016/j.tvir.2022.100818>
- Karbownik, M. S., Wiktorowska-Owczarek, A., Kowalczyk, E., Kwarta, P., Mokros, Ł., & Pietras, T. (2016). Board game versus lecture-based seminar in the teaching of pharmacology of antimicrobial drugs—a randomized

- controlled trial. *FEMS Microbiology Letters*, 363(7), fnw045. <https://doi.org/10.1093/femsle/fnw045>
- Khalidi, A., Bouzidi, R., & Nader, F. (2023). Gamification of e-learning in higher education: a systematic literature review. *Smart Learning Environments*, 10(1). <https://doi.org/10.1186/s40561-023-00227-z>
- Killian, J. K., Walker, R. J., Suuriniemi, M., Jones, L., Scurci, S., Singh, P., Cornelison, R., Harmon, S., Boisvert, N., Zhu, J., Wang, Y., Bilke, S., Davis, S. M., Giaccone, G., Smith, W. L., & Meltzer, P. S. (2010). Archival Fine-Needle Aspiration Cytopathology (FNAC) samples. *The Journal of Molecular Diagnostics*, 12(6), 739–745. <https://doi.org/10.2353/jmoldx.2010.090238>
- Kim, J., Roth, E. G., Carlisle, K., Munir, K. M., Fletke, K. J., Slejko, J. F., Mullins, C. D., & Hu, Y. (2023). Eliciting Low-Risk Thyroid Cancer Treatment Preferences using Clinical Vignettes: A Pilot Study. *Endocrine Practice*. <https://doi.org/10.1016/j.eprac.2023.04.008>
- Lampropoulos, G., & Sidiropoulos, A. (2024). Impact of gamification on students' learning outcomes and academic performance: a longitudinal study comparing online, traditional, and gamified learning. *Education Sciences*, 14(4), 367. <https://doi.org/10.3390/educsci14040367>
- Le, H. N., Cuenen, A., Trinh, T. A., Janssens, D., Wets, G., & Brijs, K. (2024). Investigating the immediate and mid-term effect of a gamified e-learning platform for the enhancement of traffic knowledge and skills among Vietnamese adolescents operating powered two-wheelers. *Journal of Safety Research*, 90, 62–72. <https://doi.org/10.1016/j.jsr.2024.06.005>
- Li, M., Meheus, F., Polazzi, S., Delafosse, P., Borson-Chazot, F., Seigneurin, A., Simon, R., Combes, J., Maso, L. D., Colonna, M., Duclos, A., & Vaccarella, S. (2023). The Economic Cost of Thyroid Cancer in France and the Corresponding Share Associated With Treatment of Overdiagnosed Cases. *Value in Health*. <https://doi.org/10.1016/j.jval.2023.02.016>
- Liu, C., Patel, R., Ogunjinmi, B., Briffa, C., Allain-Chapman, M., Coffey, J., Kallam, N., Leung, M. Y. T., Lim, A., Shamsad, S., El-Sharnouby, F., Tsang, E. K., Whitehead, J., Bretherton, J., Ramsay, L. C., & Shelmerdine, S. C. (2020). Feasibility of a paediatric radiology escape room for undergraduate education. *Insights Into Imaging*, 11(1). <https://doi.org/10.1186/s13244-020-00856-9>
- Maliki. (2017, April 26). GET TO KNOW US MEDICAL LABORATORY TECHNOLOGIST - PORTAL MyHEALTH. <http://www.myhealth.gov.my/en/medical-laboratory-technologist/>
- McGary, C. T. (2018). Educational Case: Medullary Thyroid Carcinoma. *Academic Pathology*, 5, 237428951877017. <https://doi.org/10.1177/2374289518770173>
- McHenry, W. K., & Makarius, E. E. (2023). Understanding gamification experiences with the benefits dependency network lens. *Computers and Education Open*, 4, 100123. <https://doi.org/10.1016/j.caeo.2023.100123>
- Monappa, V., & Kudva, R. (2017). Cytomorphologic diversity of papillary thyroid carcinoma. *Journal of Cytology/Journal of Cytology*, 34(4), 183. https://doi.org/10.4103/joc.joc_164_16
- Murillo-Zamorano, L. R., López-Sánchez, J. F., López-Rey, M. J., & Bueno-Muñoz, C. (2023). Gamification in higher education: The ECon+ star battles. *Computers & Education*, 194, 104699. <https://doi.org/10.1016/j.compedu.2022.104699>
- Myung, S., Lee, C. G., Lee, J., Kim, D., & Kim, H. C. (2017). Risk Factors for Thyroid Cancer: A Hospital-Based Case-Control Study in Korean Adults. *Cancer Research and Treatment*, 49(1), 70–78. <https://doi.org/10.4143/crt.2015.310>
- NCI Dictionary of Cancer Terms. (n.d.). National Cancer Institute. <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/thyroid-gland>
- Ogino, H., & Matsuguma, H. (2024). Effective implementation of feedback in gamification: Verifying the utility of feedback to induce behavior. *Kyushu University, Graduate School of Design*. https://www.jstage.jst.go.jp/article/adada/28/2/28_9/_pdf
- Pitt, S. C., Zanocco, K., & Sturgeon, C. (2022). The Patient Experience of Thyroid Cancer. *Endocrinology and Metabolism Clinics of North America*, 51(4), 761–780. <https://doi.org/10.1016/j.ecl.2022.04.002>
- Rosa-Castillo, A., García-Pañella, O., Maestre-Gonzalez, E., Pulpón-Segura, A. M., Roselló-Novella, A., & Solà-Pola, M. (2022). Gamification on Instagram: Nursing students' degree of satisfaction with and perception of

- learning in an educational game. *Nurse Education Today*, 118, 105533. <https://doi.org/10.1016/j.nedt.2022.105533>
- Rua, R., Argüelles, S., Carreras, O., & Ojeda, M. D. (2022). Selenium, selenoproteins and cancer of the thyroid. *Journal of Trace Elements in Medicine and Biology*, 76, 127115. <https://doi.org/10.1016/j.jtemb.2022.127115>
- Ruiz-Bañuls, M., Trigueros, I. M. G., Rovira-Collado, J., & Rico-Gómez, M. L. (2021). Gamification and transmedia in interdisciplinary contexts: A didactic intervention for the primary school classroom. *Heliyon*, 7(6), e07374. <https://doi.org/10.1016/j.heliyon.2021.e07374>
- Sánchez-Martín, J., Cañada, F. C., & Acedo, M. a. D. (2017). Just a game? Gamifying a general science class at university. *Thinking Skills and Creativity*, 26, 51–59. <https://doi.org/10.1016/j.tsc.2017.05.003>
- Sandrone, S., & Carlson, C. (2021). Gamification and game-based education in neurology and neuroscience: Applications, challenges, and opportunities. *Brain Disorders*, 1, 100008. <https://doi.org/10.1016/j.dscb.2021.100008>
- Sharma, M., & Cetera, B. (2020). Thyroid disease and surgery. *Anaesthesia & Intensive Care Medicine*, 21(11), 558–571. <https://doi.org/10.1016/j.mpaic.2020.09.001>
- Tan, L., Tan, Y., & Tan, S. (2020). Diagnostic accuracy and ability to reduce unnecessary FNAC: A comparison between four Thyroid Imaging Reporting Data System (TI-RADS) versions. *Clinical Imaging*, 65, 133–137. <https://doi.org/10.1016/j.clinimag.2020.04.029>
- Thakur, M. (n.d.). Sample Size Formula. Wall Street Mojo. <https://www.wallstreetmojo.com/sample-size-formula/>
- Thomas, N. J., Baral, R., Crocco, O. S., & Mohanan, S. (2023). A framework for gamification in the metaverse era: How designers envision gameful experience. *Technological Forecasting and Social Change*, 193, 122544. <https://doi.org/10.1016/j.techfore.2023.122544>
- Thyroid Cancer | CDC. (n.d.). CDC. <https://www.cdc.gov/cancer/thyroid/index.htm#:~:text=How%20Can%20I%20Reduce%20My,around%20the%20head%20and%20neck>
- Thyroid Cancer: Symptoms, Causes, Diagnosis, Treatment | Gleneagles Hospitals. (n.d.). Gleneagles. <https://www.gleneagles.com.my/medical-specialties/oncology/thyroid-cancer#:~:text=In%20Malaysia%2C%20the%20incidence%20of,with%20an%20excellent%20cure%20rate>
- Thyroid Cancer Treatment (PDQ®)—Patient Version. (2023, March 16). National Cancer Institute. <https://www.cancer.gov/types/thyroid/patient/thyroid-treatment-pdq#:~:text=Thyroid%20cancer%20is%20a%20disease,the%20risk%20of%20thyroid%20cancer>
- Thyroid Cytopathology. (n.d.). Cellnetpathology.com. <https://www.cellnetpathology.com/thyroid-cytopathology>
- Thyroid fine needle aspiration biopsy. (2019, November 19). Johns Hopkins Medicine. <https://www.hopkinsmedicine.org/health/treatment-tests-and-therapies/thyroid-fine-needle-aspiration-biopsy>
- Thyroglossal Duct Cysts and Sublingual Thyroid. (n.d.). JaypeeDigital. <https://www.jaypeedigital.com/book/9789351528067/chapter/ch4>
- Tseng, C., Wei, C., Kuo, C., Yan, S., Chen, P., Lien, W., Chang, S., Su, T., & Tseng, Y. (2008). Fine Needle Aspiration Cytology of Thyroid Nodules: Evaluation of Diagnostic Accuracy. *Tzu Chi Medical Journal*. [https://doi.org/10.1016/s1016-3190\(08\)60054-3](https://doi.org/10.1016/s1016-3190(08)60054-3)
- Van Houten, P., Netea-Maier, R. T., & Smit, J. (2022). Differentiated thyroid carcinoma: An update. *Best Practice & Research Clinical Endocrinology & Metabolism*, 37(1), 101687. <https://doi.org/10.1016/j.beem.2022.101687>
- Walsh, N. (2022, March 9). Thyroid Cancer Prevention: Top 5 Things You Can Do. Clayman Thyroid Center. <https://www.thyroidcancer.com/blog/thyroid-cancer-prevention-top-5-things-you-can-do>
- Westenhaver, Z. K., Africa, R., Zimmerer, R. E., & McKinnon, B. J. (2021). Gamification in otolaryngology: A narrative review. *Laryngoscope Investigative*

Otolaryngology, 7(1), 291–298.
<https://doi.org/10.1002/lio2.707>

Wu, Z., Heinegård, D., Li, W., Wang, W., Chen, L., Yao, Y., & Wang, Y. (2021). Which is preferred for initial treatment of papillary thyroid cancer, total thyroidectomy or lobotomy? *Cancer Medicine*, 10(5), 1614–1622.
<https://doi.org/10.1002/cam4.3743>

Youssef, M. R., Reisner, A. S. C., Attia, A. S., Hussein, M., Omar, M. A., LaRussa, A., Galvani, C. A., Aboueisha, M., Abdelgawad, M. A., Toraih, E. A., Randolph, G. W., & Kandil, E. (2021). Obesity and the prevention of thyroid cancer: Impact of body mass index and weight change on developing thyroid cancer – Pooled results of 24 million cohorts. *Oral Oncology*, 112, 105085.
<https://doi.org/10.1016/j.oraloncology.2020.105085>

Zhang, J., Zhang, J., & Han, J. (2022). Advantages and clinical significance of enhanced CT combined with BRAF V600E gene detection in the diagnosis of papillary thyroid carcinoma. *Medical Engineering & Physics*, 110, 103862.
<https://doi.org/10.1016/j.medengphy.2022.103862>

Zhang, L., Xu, S., Cheng, X. W., Wu, J., Wu, L., Wang, Y., Wang, X., Bao, J., & Yu, H. (2022). Curcumin induces autophagic cell death in human thyroid cancer cells. *Toxicology in Vitro*, 78, 105254.
<https://doi.org/10.1016/j.tiv.2021.105254>

Zhang, Z., Chhieng, D. C., Harshan, M., Zheng, X., & Zakowski, M. F. (2019).

Cytological features of noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). *Journal of the American Society of Cytopathology*, 8(1), 5–10. <https://doi.org/10.1016/j.jasc.2018.07.007>

Zhu, Y., Song, Y., Xu, G., Fan, Z., & Ren, W. (2020). Causes of misdiagnoses by thyroid fine-needle aspiration cytology (FNAC): our experience and a systematic review. *Diagnostic Pathology*, 15(1).
<https://doi.org/10.1186/s13000-019-0924-z>