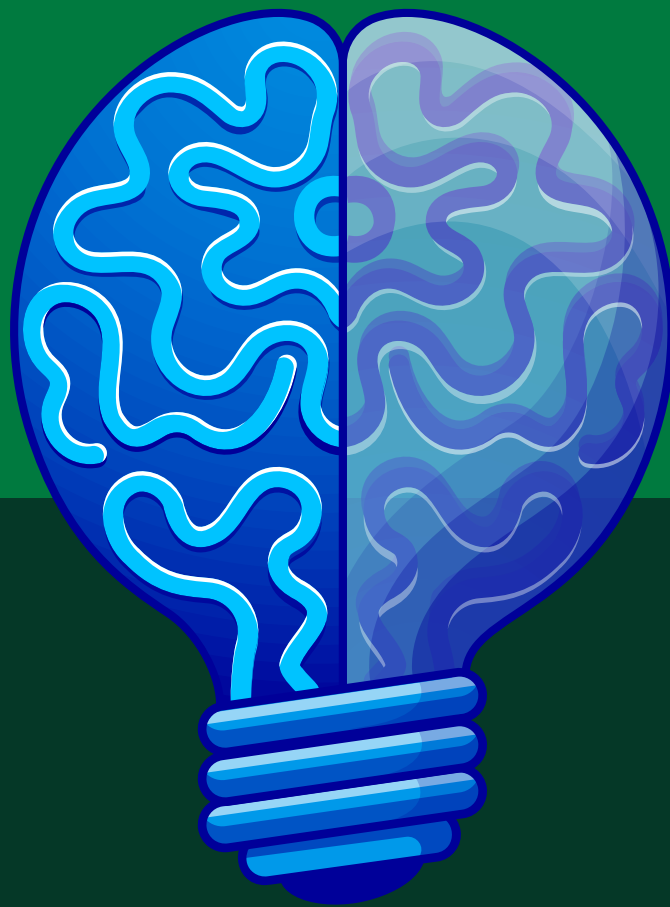


FACULTY OF  
APPLIED SCIENCES

UNIVERSITI TEKNOLOGI MARA  
PERAK BRANCH

# SCIENTIFIC PROJECT COLLOQUIUM 2025



BIOLOGY ~ CHEMISTRY ~ PHYSICS

Final Year Project Colloquium 2025  
Faculty of Applied Sciences,  
Universiti Teknologi MARA,  
Perak Branch Tapah Campus,  
35400 Tapah Road, Perak, Malaysia.

Colloquium date 24<sup>th</sup> January 2025  
Publication date 31<sup>st</sup> October 2025

Proceedings of Extended Abstracts

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e-1.S.B.N: 978-629-97630-6-2

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## **Preface**

The Scientific Project Colloquium offers a platform for publishing Diploma Science final year projects (FYP). The objective is to effectively distribute research findings throughout all scientific disciplines. The primary objective of including final year projects into the course curriculum is to encourage students to put their theoretical knowledge into practical applications.

We would like to express our gratitude to our primary establishment, the Faculty of Applied Sciences and Universiti Teknologi MARA, Perak Branch, for their invaluable assistance.

Lastly, we would like to express our gratitude to all of the authors for the tremendous help in preparing the articles, without which this undertaking would not have been completed.

## **Editors**

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October 2025

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# MODELING SILICON NITRIDE (SiNx) ANTI-REFLECTIVE COATINGS FOR SILICON SOLAR CELLS VIA RAY-TRACING SIMULATION

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**Abstract:** This research focuses on optimizing double-layer anti-reflective coatings (ARC) combined with front surface upright pyramid texturing to enhance the optical performance of silicon solar cells. Simulations were conducted using the PV Lighthouse wafer ray tracer software to evaluate a light trapping (LT) scheme applied to a 100  $\mu\text{m}$  thin crystalline silicon (c-Si) base under the AM1.5G solar spectrum at normal incidence, to improve broadband absorption in the 300–1200 nm wavelength range. Double-layer ARC of Silicon Nitride (SiNx) and silicon dioxide (SiO<sub>2</sub>) with varying thicknesses were examined. The findings demonstrate that the double-layer ARC with an upright pyramid front surface, consisting of 80 nm SiNx and 60 nm SiO<sub>2</sub>, yielded the highest performance, achieving a photocurrent density of 40.82 mA/cm<sup>2</sup>. This represents a  $J_{\text{max}}$  enhancement of 65.33%, indicating that the combination of optimized ARC thickness and front surface texturing provides the most effective light trapping and absorption enhancement.

**Keywords:** *Light trapping, Solar cell, Anti-reflective coating (ARC), Ray tracing, Photocurrent density*

## INTRODUCTION

Most electricity in the world was taken by several forms such as nuclear resources, fossil fuels and renewable resources. Some examples of fossil fuels and nuclear resources are coal, oil, and natural gas while examples for renewable resources are solar, wind, geothermal and hydropower (Zambree et al., 2023). All these renewable resources have been thoroughly investigated and very useful in energy production. It is because renewable resources are widely available, free and are not harmful to the environment (Amalathas & Alkaisi, 2019). Among all these renewable resources, solar energy is recognized as the clean renewable energy source because it is gained from the sunlight. Solar cell or called a photovoltaic cell is a device that can generate electrical energy from the light energy through the photovoltaic effect (Zambree et al., 2023). Solar energy can replace fossil fuels to generate electricity because it requires low cost and more efficient in its production (Jamaluddin et al., 2022). Solar cells produce electricity without the need for any moving parts or electricity generators or chemical reactions such as batteries or fuel cells (Zambree et al., 2023).

Like other technologies, solar cells must overcome several obstacles and problems to provide consumers with reasonably priced electricity. One of the problems of solar cells is optical losses or reflection losses. When the light energy hits the surface of the solar cell, some part of the light will absorb into the cell while other parts will reflect. To overcome this problem, a light trapping (LT) scheme was applied to the solar cell which included the surface texturing and anti-reflective coating (ARC) to reduce the reflection of light and increase the performance of the solar cell. Anti-reflective coating (ARC) is one of the efficient ways to prevent the reflection of light from the surface of the cell exactly as its name suggests. ARC used to increase light absorption and performance of the solar cell. They are made up of two layers either single layer and double layer ARC of precisely selected thickness dielectric substance.

However, the number of anti-reflective coating compounds available is limited due to refractive index limits. For example, SiO<sub>2</sub>, MgF<sub>2</sub>, SiNx, Al<sub>2</sub>O<sub>3</sub>, TiO<sub>2</sub>, and ZnS are the common materials that have been used for ARC (Salleh, 2022). Besides, one popular method to produce light trapping is to change the angle at which light travels in the solar cell by incidentally hitting an angled surface (Zambree et al., 2023). Planar and pyramid are the type of surface that is regularly used for the front surface of the solar cell. Pyramid surface which is textured surface is the best type of surface to reduce reflection which makes the optical path length increase (Zambree et al., 2023).

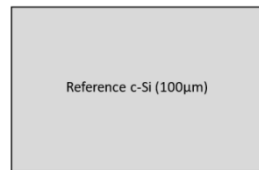
In this paper, ray tracing of LT schemes of double-layer ARC of Silicon Nitride (SiNx) and silicon dioxide (SiO<sub>2</sub>) in thin c-Si solar cells (with 100  $\mu\text{m}$  thickness) is investigated. Four LT schemes with difference thickness, each on random upright pyramid front surface are studied. Effects of these LT schemes towards reflection, transmission and absorption of the incident light in the thin c-Si absorber are analysed.

## METHODOLOGY

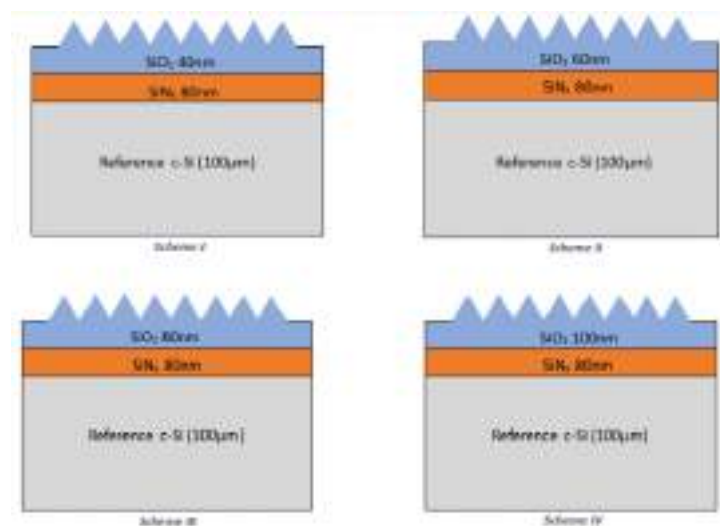
In this work, wafer ray tracer software that is called PV Lighthouse is used to investigate ray tracing of LT schemes

in thin c-Si solar cells (with a thickness of 100  $\mu\text{m}$ ). This software has many functions which can determine the photogenerated current density in a solar cell or modelling the structure using a selected light source. In addition, it shows a generated curve of reflection, absorption and transmission which make it easier to plot a graph. By using this software, it produces more accurate and detailed results. The solar spectrum used is AM1.5G at zero incidence angle which is normal to the surface of the solar cell. For ray tracing, a maximum of 50,000 rays can be used where 5,000 rays are used per run. The range of wavelengths is from the minimum wavelength of 300 nm to the highest wavelength of 1200 nm with a wavelength interval of 20 nm is used (Jamaluddin et al., 2022).

In this research, the substrate of the solar cell is a thin crystalline silicon, c-Si with a random planar surface which is used as reference shown in Figure 1. Materials for anti-reflective coating (ARC) were used are  $\text{SiN}_x$  and  $\text{SiO}_2$  where  $\text{SiN}_x$  is placed at the top layer while  $\text{SiO}_2$  is placed at the bottom layer. Figure 2 indicated a double layer ARC of  $\text{SiN}_x$  and  $\text{SiO}_2$  with thickness of  $\text{SiN}_x$  was fixed at 80 nm while  $\text{SiO}_2$  with various thickness that starts at 40 nm, 60 nm, 80 nm, and 100 nm. Solar cells are improved by adding the upright pyramid front surface to observe the performance of light absorption. The angle of the pyramid is  $54.74^\circ$ , with height 3.536  $\mu\text{m}$  and width 5  $\mu\text{m}$  which was set in the software.



**Figure 1** Schematic diagram of c-Si (with thickness 100  $\mu\text{m}$ ) without LT scheme as reference



**Figure 2** Schematic diagram of double layer ARC with upright pyramid front surface on substrate silicon

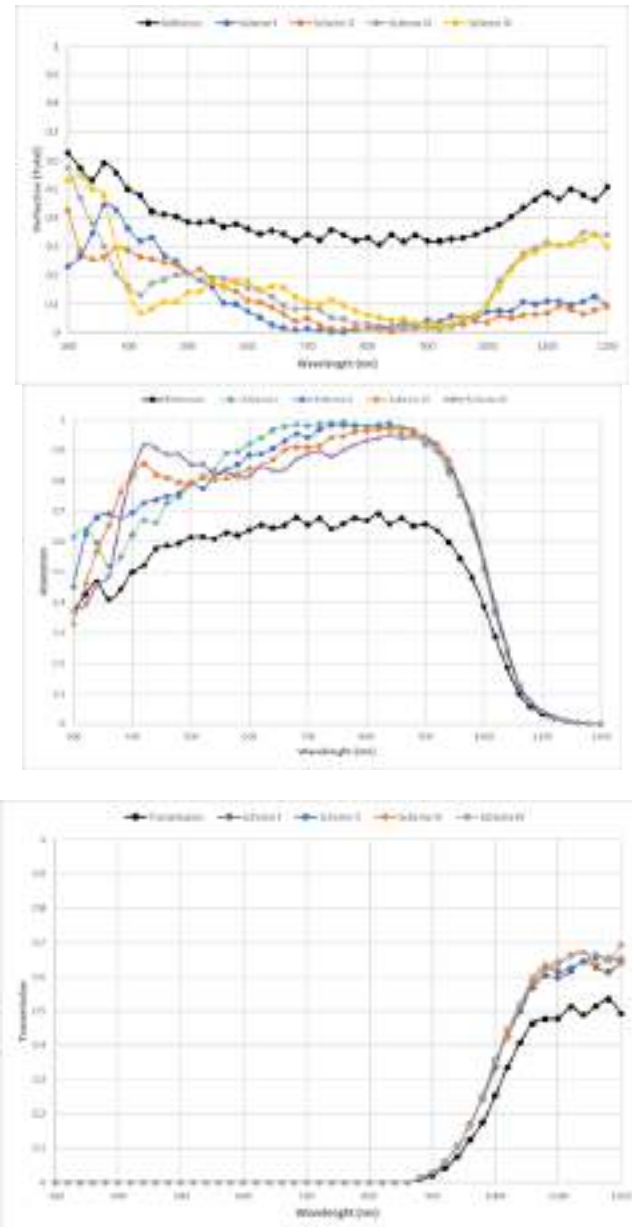
In the simulation, total reflection, absorption, transmission, and photocurrent density,  $J_{\text{max}}$  of double layer ARC in thin c-Si wafers were analysed. Besides, the value of  $J_{\text{max}}$  is used to observe the performance of solar cells. The percentage of  $J_{\text{max}}$  enhancement was calculated by using the formula as shown in Equation (1).

$$J_{\text{max}} \text{ enhancement} = [(J_{\text{max}} \text{ LT scheme} - J_{\text{max}} \text{ ref})/J_{\text{max}} \text{ ref}] \times 100 \dots(1)$$

## FINDINGS

The curves for reflection, absorption and transmission of a thin crystalline silicon, c-Si solar cell are included in Figure 3(a)–(c). A reference graph is included in order to analyse and compare the differences between the four LT schemes. Reference solar cells show high broadband reflection throughout the 300–1200 nm the wavelength region. This is explained by the sudden shift in refractive index ( $n$ ) that occurs when incident light moves from air ( $n=1$ ) to c-Si ( $n=3.5$ ). With LT Scheme (upright pyramid; height = 5mm, base angle =  $54.74^\circ$ ) reflection from wavelength 300 nm to 950 nm is lower and reduces even more compared to LT schemes before because of increased light scattering at the interface between the air and the pyramid. High oblique angles cause the incident light to be scattered into the thin c-Si. A portion of the scattered light is eventually trapped in the c-Si absorber after passing through total internal reflection.

Due to the fact that it does not pair up with any type of LT scheme, the standard reference of c-Si in Figure 3b) has a very low broadband of absorption, increasing to only 40% at wavelength 420 nm to 860 nm. Broadband light absorption also increases significantly due to the increased light scattering from 300 to 600 nm compared to reference. From the optical results above, the device structure that gives the optimum result is the Scheme I. Figure 3(c). The transmission profile on an identical front surface remains constant regardless of thickness. However, it is clear that an upright pyramid front surface has a significant effect on lowering light transmission in solar cell schemes. The value of reduction of transmission is almost 25 % compared to reference. Transmission of the short wavelength reduces to 0 due to the presence of ARC. However, at long wavelengths (above 900 nm) the transmission for all LT schemes is increasing, reaching 28% and 10 % at 1,100 nm for front planar and upright pyramid front surfaces, respectively. This is due to indirect bandgap semiconductor in Si so there is a long tail in absorption out to long wavelength resulting in higher transmittance. (Zambree et al., 2023)



**Figure 3** a) Reflection b) Absorption c) Transmission curves for silicon solar cells with SiNx ARC and different thickness of 40 nm, 60 nm, 80 nm, 100 nm SiO<sub>2</sub> ARC. Reference curve of silicon solar cells (black curve) included for comparison.

**Table 1** Summary of  $J_{\max}$  of thin c-Si (with 100  $\mu\text{m}$  thickness) with incremental LT schemes.  $J_{\max}$  reference c-Si is included for comparison.

| LT Scheme   | $J_{\max}$ (mA/cm <sup>2</sup> ) | Enhancement (%) |
|---|----------------------------------|-----------------|
| Reference c-Si (thickness = 100nm)  | 24.69                            | -               |
| <b>Scheme I:</b> Upright Pyramid (height = 5mm, base angle = 54.74°) with SiO <sub>2</sub> /SiN <sub>x</sub> (SiO <sub>2</sub> = 40nm, SiN <sub>x</sub> = 80 nm)  | 40.76                            | 65.08           |
| <b>Scheme II:</b> Upright Pyramid (height = 5mm, base angle = 54.74°) with SiO <sub>2</sub> /SiN <sub>x</sub> (SiO <sub>2</sub> = 60nm, SiN <sub>x</sub> = 80 nm) | 40.82                            | 65.33           |
| <b>Scheme III:</b> Upright Pyramid (height =5mm, base angle = 54.74°) with SiO <sub>2</sub> /SiN <sub>x</sub> (SiO <sub>2</sub> = 80nm, SiN <sub>x</sub> = 80 nm) | 40.79                            | 65.21           |
| <b>Scheme IV:</b> Upright Pyramid (height =5mm, base angle = 54.74°) with SiO <sub>2</sub> /SiN <sub>x</sub> (SiO <sub>2</sub> = 100 nm, SiN <sub>x</sub> = 80nm) | 40.77                            | 65.12           |

Table 1 displays the  $J_{\max}$  measured value of the thin c-Si (100  $\mu\text{m}$  thickness) using incremental LT schemes. Comparing Scheme I (40 nm) with the c-Si reference, where  $J_{\max}$  is equal to 24.69 mA/cm<sup>2</sup>.  $J_{\max}$  increased to 40.76 mA/cm<sup>2</sup>, indicating a 65.08% improvement. In addition, at some point in the wavelength range, light absorption within the thin c-Si begins to increase using Scheme II (60 nm) It is because the best achievement of highest absorption, lower reflection and transmission. This condition is known as the finest condition of the efficiency of solar cells. At 40.82 mA/cm<sup>2</sup>,  $J_{\max}$  increased, indicating a 65.33% improvement. The thin c-Si's high optical light absorption increased after Scheme III (80 nm) was used. At 40.79 mA/cm<sup>2</sup>, the  $J_{\max}$  tends to rise for an enhancement of approximately 65.21%. In the end, Scheme IV's high optical wavelength of 100 nm for light absorption gained roughly 40.77 mA/cm<sup>2</sup> with a 65.12% enhancement.

## CONCLUSIONS

The ray tracing of LT schemes in 100 $\mu\text{m}$  thick thin c-Si solar cells with SiN<sub>x</sub> anti-reflective coating (ARC) has been studied in this work. Research has been done on the impact of surface texturing of the front layer of film on silicon solar cells' performance. For the double-layer anti-reflective coating (ARC), SiO<sub>2</sub>/SiN<sub>x</sub> was used. The study focused on four distinct light trapping techniques, derived from multiples of the SiO<sub>2</sub> thickness values of 40 nm, 60 nm, 80 nm, and 100 nm. This simulation demonstrates that among LT schemes, double-layer ARC with an upright pyramid front surface of 80 nm SiN<sub>x</sub> and 60 nm SiO<sub>2</sub> thickness gave the best improvement in current density of 40.82 mA/cm<sup>2</sup>. This showed the  $J_{\max}$  enhancement is 65.33%.

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# EFFECTS OF DIFFERENT LIGHT WAVELENGTHS ON THE GROWTH OF WATER SPINACH (*IPOMOEA AQUATICA*)

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**Abstract:** This study explores the effect of different LED light wavelengths on the growth of water spinach (*Ipomoea aquatica*). Over an eight-day period, plants were exposed to red, blue, white, and green LEDs, and their height and leaf development were monitored. Red light produced the greatest height increase (4.7 cm), while blue and white lights supported balanced leaf growth, with three leaves each by Day 8. Green light resulted in the least growth. These results highlight the importance of light spectrum in shaping plant growth and suggest practical applications for optimizing sustainable indoor agriculture.

**Keywords:** *Water spinach, LED light wavelengths, Plant growth, Sustainable indoor agriculture*

## INTRODUCTION

Light is one of the most critical environmental factors influencing plant growth and development. Beyond its role as the primary energy source for photosynthesis, light also regulates several physiological processes such as photomorphogenesis, stomatal activity, circadian rhythm, and hormonal regulation (Hogewoning et al., 2010). The spectrum, intensity, and duration of light exposure determine how efficiently plants capture energy and how they shape their morphology. Understanding these interactions is especially relevant in controlled-environment agriculture (CEA), where artificial lighting systems are increasingly used to enhance crop productivity and sustainability (Bantis et al., 2018).

Plants respond differently to specific regions of the light spectrum due to the presence of specialized photoreceptors. Blue light with a wavelength of 400–500 nm plays an essential role in regulating leaf expansion, chlorophyll synthesis, and stomatal opening. It promotes compact growth, higher chlorophyll content, and efficient photosynthesis in leafy vegetables such as spinach and lettuce (Hasan et al., 2017; Johkan et al., 2012). However, excessive blue light may reduce stem elongation and leaf size, suggesting that optimal levels are required for balanced development. Meanwhile red light with a wavelength of 600–700 nm is vital for photosynthesis, seed germination, stem elongation, and flowering (Lin et al., 2020). When combined with blue light, red light enhances leaf size, biomass accumulation, and balanced morphology (Johkan et al., 2012). Green light with a wavelength in between 495–570 nm, recently shows evidence that suggests it can penetrate deeper into the leaf canopy, supporting photosynthesis in lower tissues where red and blue light cannot reach effectively (Kim et al., 2004). Although green light alone does not maximize growth, its integration into a full-spectrum regime may improve overall plant performance, particularly in dense plantings. Lastly, white light, which combines red, blue, green, and sometimes far-red wavelengths, closely mimics natural sunlight and provides a balanced spectrum for plant development. While it may not maximize any single growth parameter, white LED light supports moderate photosynthesis, stem elongation, and leaf expansion, making it a practical choice in small-scale and educational settings (Bourget, 2008).

The rise of light-emitting diode (LED) technology has revolutionized plant growth studies by offering spectrum-specific control, energy efficiency, and longer operational lifespans compared to traditional light sources (Massa et al., 2008). This advancement has allowed researchers to design and optimize traits such as plant height, leaf morphology, and biomass accumulation (Hasan et al., 2017). Despite this progress, many studies have focused on model crops such as lettuce and kale, while less attention has been given to leafy vegetables of regional importance, such as water spinach. Water spinach is widely cultivated in Asia and valued for its nutritional content, but its responses to specific light spectra remain underexplored.

Therefore, this study examines the effects of different LED light wavelength (red, blue, green, and white) on the growth performance of water spinach. By linking plant growth responses with physical concepts such as photon energy and light absorption, this research not only contributes to the understanding of plant–light interactions but also provides insights into the optimization of artificial lighting strategies for sustainable indoor agriculture.

## METHODOLOGY

Water Spinach was chosen for its rapid growth and sensitivity to environmental changes, making it suitable for short-term light experiments. Seeds were obtained from a certified agricultural supplier and soaked in water for 12 hours to promote uniform germination. Then, about 20 seeds were planted in each container. The elongation and the growth of water spinach was an average data obtained from each container. The soil used is made up from a 1:1 mixture of coconut coir and organic compost, providing good aeration, water retention, and nutrients. Each container was placed inside a light-isolation box to prevent contamination between treatments as shown in Figure 1. Four compartments were equipped with red, blue, green, or white LEDs. The environment was maintained at ~26°C, and each plant was watered with 10 mL of distilled water twice daily. No fertilisers were applied to ensure that light was the only variable affecting growth. Four LED types were tested: red (660 nm), blue (450 nm), green (520 nm), and white (full spectrum). Lamps were positioned 25 cm above the plants with equal power output to maintain uniform intensity. Plants were exposed to a 16 h light/8 h dark cycle daily, controlled by a digital timer. Each compartment was lined with reflective material to maximise light distribution and eliminate interference from external light sources.



**Figure 1** (a) and (b) is the experimental set up for measuring the plant growth for different light

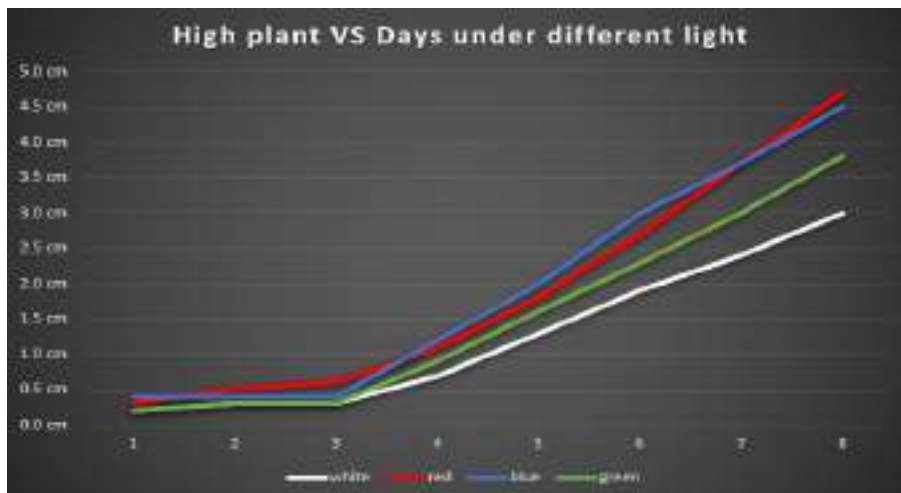
## FINDINGS

The growth of water spinach under different LED light treatments revealed significant variations in plant height across the eight-day period as shown in Table 1. From the data, it is observed that red and blue light treatments produced the greatest increases in height compared to white and green light. At day 8, plants grown under red light reached 4.7 cm, slightly exceeding those under blue light at 4.5 cm. Meanwhile, plants under white (3.0 cm) and green light (3.8 cm) showed comparatively slower growth. The growth observed under red light is consistent with the role of red wavelengths in stimulating phytochromes, which regulate stem elongation and biomass accumulation (Lin et al., 2020). From the data, blue light also contributed substantially to height gain, specifically after day 4, reflecting its role in chlorophyll production, stomatal regulation, and efficient photosynthesis (Johkan et al., 2012). The data for green light treatment shows the lowest growth performance which is aligning with reports that plants reflect much of the green spectrum, reducing its effectiveness for photosynthesis (Kim et al., 2004) while white light, as a full-spectrum treatment, provided intermediate results, confirming its ability to support general development but not maximise any single growth parameter (Bourget, 2008).

**Table 1** Plant height of water spinach (*Ipomoea aquatica*) under different LED light treatments

| Day | White (cm) | Red (cm) | Blue (cm) | Green (cm) |
|-----|------------|----------|-----------|------------|
| 1   | 0.2        | 0.3      | 0.4       | 0.2        |
| 2   | 0.3        | 0.5      | 0.4       | 0.3        |
| 3   | 0.3        | 0.6      | 0.4       | 0.3        |
| 4   | 0.7        | 1.1      | 1.2       | 0.9        |
| 5   | 1.3        | 1.8      | 2.0       | 1.6        |
| 6   | 1.9        | 2.7      | 3.0       | 2.3        |
| 7   | 2.4        | 3.7      | 3.7       | 3.0        |
| 8   | 3.0        | 4.7      | 4.5       | 3.8        |

The growth trends of water spinach under different LED light treatments are shown in Figure 2. Across the 8-day period, plant height increased steadily under all light conditions, but the rate of elongation varied significantly depending on the wavelength provided. From this figure, after 8 days of observation, red and blue LED lights promoted the highest growth rates, with final plant heights of 4.7 cm and 4.5 cm, respectively. This indicates that both wavelengths are highly effective in stimulating vegetative growth, though red light showed a slight advantage in overall elongation. The pronounced effect of red light supports its role in activating phytochromes, which regulate stem elongation and photosynthetic efficiency (Lin et al., 2020). Green light produced moderate growth, with plants reaching 3.8 cm by Day 8. Although green wavelengths are largely reflected by plant tissues, their ability to penetrate deeper into the canopy may provide supplementary photosynthesis in lower leaves (Kim et al., 2004). However, compared to red and blue light, the growth-promoting effect of green was weaker.



**Figure 2** The plant height versus different light used

## CONCLUSIONS

This study demonstrates that the growth of *water spinach* is strongly influenced by the wavelength of LED light. Among the treatments, red light produced the greatest stem elongation (4.7 cm), followed closely by blue light (4.5 cm), while green light supported moderate growth (3.8 cm) and white light resulted in the lowest growth (3.0 cm). These findings highlight the role of red and blue wavelengths in promoting rapid vegetative development through the activation of specific photoreceptors, whereas green and white lights were less effective. Based on the results, it can be suggested that combining red and blue wavelengths, can optimize plant growth and offer practical benefits for sustainable indoor agriculture.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# DISCOVERING THERMAL PHYSICS: MALE AND FEMALE PERSPECTIVES ON LEARNING THROUGH AN INTERACTIVE CALENDAR

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**Abstract:** Thermal physics is one of the main topics in physics, especially for those who study sciences and engineering. Due to the concepts and mathematics involved in the topic, students may find it difficult and finally lose interest in studying Thermal Physics. The major objective of this project is to create a tool that will aid students in better, more enjoyable ways of understanding thermal physics. Thermal Physics Interactive calendar that includes concepts, facts, quizzes, and experiments that can try at home. Having a calendar that includes interesting thermal physics concepts may help the students to learn and gain knowledge in that area. Thirty students of the Diploma in Science from Universiti Teknologi MARA (UiTM) are involved in providing feedback. Male students find thermal physics course to be less fascinating due to the mean score for interesting being less than 4. The Spearman Rho test, using the interactive calendar, demonstrates a strong correlation between 1) preference and 2) content, with a t value of +0.937. In addition, an independent t-test reveals that there are no further significant differences in grades for male and female students in the following categories: 1) preference ( $t=0.437$ ,  $p=0.666$ ); and 2) content ( $t=0.280$ ,  $p=0.782$ ).

**Keywords:** *Thermal physics, T-test, Interactive calendar, Spearman Rho test, Students*

## INTRODUCTION

The growth rate of knowledge becomes faster and faster, and the skill update time becomes shorter and shorter (Sun et al., 2020). The amount of knowledge or information students can inhale in a classroom depends on their attitude toward learning. Students may find it difficult to learn physics because of the subject's complex concepts and mathematics. Teachers devote their all in the classroom to ensure that the students understand the content of the subject course. It is important to maintain the students' attention early on from the beginning of the semester by stimulating their interest to learn and proving the relevance of the subjects with real-life implications so that it would improve the students' learning satisfaction (Musa et al., 2018). Students will learn more if their interest in the subject is increased. The attention of the learners will also rise with the addition of fresh developments in teaching materials. The use and integration of technology should be directed by the educational needs to optimize the learning outcomes, where specific roles and objective prerequisites for optimal results can be determined (Tuma, 2021). The role of teachers also helps the students to overcome the challenges that might appear in the classroom. Therefore, flexibility in teaching is a must, where teachers applying training methods that involve two-way communications will enable you to make quick adjustments in processes and approaches (Senthamarai, 2018). This falls under the category of interactive learning. Thus, interactive learning is a special learning, during which there is interaction between the student and the teacher, as well as between the students themselves (Orshanskyi, 2020). A single method cannot meet all of our goals nor can a single method accommodate all learning styles at once (Palaniyammal, 2018). In contrast to using traditional bookish approaches, interactive calendars can be used as a learning tool to make content more engaging. The interactive calendar offers quizzes, games, facts, and experiments that you may explore at home. People in general need a calendar to remember dates and schedule upcoming events. A calendar with fascinating thermal physics concepts may aid students in learning and expanding their understanding of the subject.

## METHODOLOGY

### 1) Preparation of the calendar

To prepare the calendar, we have to plan the topics of each month and also the activities that students can do throughout the month. Starting from January to December 2023, it covers all subtopics for thermal physics.

### 2) Survey Questionnaire

A sample of 30 students from the second semester of the Diploma in Science program—15 male and 15 females—volunteered to review and reflect on the desk calendar that had been provided to them. Several sections of the questionnaire were developed based on how the students felt about the thermal physics course and what they thought of the interactive calendar. The questionnaires included ratings of the students' satisfaction on a five-point Likert scale (1=Strongly disagree to 5=Agree).

### 3) Research Question

The following research questions were posed to guide this investigation.

1. How do students feel about the Thermal Physics course?
2. Is there any correlation between 2 categories, 1) Preference 2) Content?
3. Is there any significant difference in the mean score between male and female students towards the interactive calendar?

### 4) Research Hypothesis

The following research hypothesis was proposed to promote further research as shown in Table 1, besides the month from January to December. Each month has its own topics and activities that are attached in the calendar.

**Table 1** Table of hypotheses for both category perspective towards the interactive calendar

| Hypothesis                    | Category 1: Preference  | Category 2: Content   |
|-------------------------------|---|---|
| Null Hypothesis, $H_0$        | There is no significant difference between the 2 means of male and female students for preference | There is significant difference between 2 the means of male and female students for preference. |
| Alternative Hypothesis, $H_1$ | There is no significant difference between the 2 means of male and female students for content.   | There is significant difference between the 2 means of male and female students for content.    |

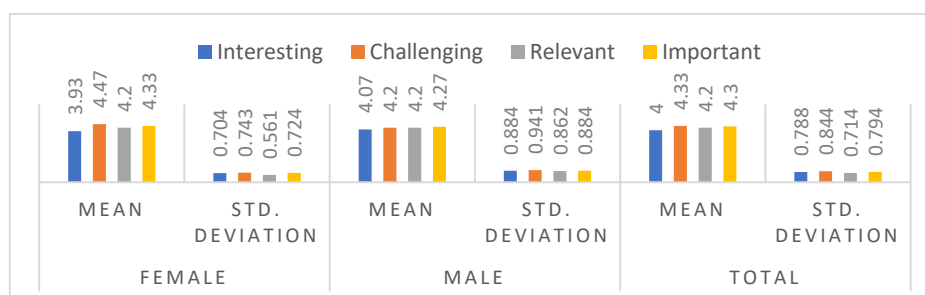
**Table 2** The topic and activity for respective month

| Month     | Topics                 | Activities  |
|-----------|------------------------|---|
| January   | Temperature            | Learning the concept of temperature and games   |
| February  | Thermal Equilibrium    | Learning the concept of thermal equilibrium and try the experiment at home                            |
| March     | Thermometer            | Learning about the thermometers and doing quizzes   |
| April     | Temperature Scale      | Learning about the thermometer scale  |
| May       | Calorimetry            | Learning the concept of calorimetry and trying the experiment at home                                 |
| June      | Heat                   | Learning the concept of heat and identifying objects that have higher kinetic energy.                 |
| July      | Specific Heat Capacity | Learning the heat capacity equation and identifying the specific heat capacity of different substance |
| August    | Latent Heat            | Learning about the concept of Latent heat and equation involves.                                      |
| September | Phase Change           | Learning about the phase change and identify the process  |
| October   | Conduction             | Learning about the conduction equation  |
| November  | Convection             | Learning about the convection process   |
| December  | Radiation              | Learning about the radiation equation.  |

## FINDINGS

### 1) Survey on Thermal Physics Courses

Started with the survey of the thermal physics courses themselves. We need to know students' perspectives on the subject in order to identify the problem that students face in learning the subject. There are 4 main components which are: 1) Interesting 2) Challenging 3) Relevant and 4) Important. According to a Likert scale (1=Strongly Disagree to 5=Strongly Agree), the students will submit their input, and the results will be translated into mean and standard deviation form.



**Figure 1** The graph for mean and standard deviation male and female based on interesting, challenging, relevant and important.

The mean value for male and female students is marginally different, according to the Figure 1 above. Given that female students scored higher on the challenging scale (M=4.47, SD=0.743) and male students scored similarly high (M=4.20, SD=0.941), both genders agree that the Thermal Physics course is difficult. Most likely because of how challenging it is for students to captivate the course-related concept and equation. The course is rated as the most important by male students (M=4.27, SD=0.884), on average. Male students may be more conscious of the significance of the course. Under the item interesting, both male and female students received the lowest mean scores (male M=4.07 and female M=3.93). As a result, both male and female students find this thermal physics course to be less fascinating. Hopefully, the students' perspective on thermal physics will change because of this research.

## 2) Survey on Thermal Physics Calendar

The same goes for the survey on the usage of the interactive calendar using the Likert Scale (1=Strongly Disagree to 5=Strongly Agree) for 9 different elements. The element is divided into two groups, which are 1) preference and 2) content of the calendar. Where the preference is based on design, quality, features, new invention and impression. While for content cover, it is usefulness, informative, interactive, and recommended.

### a) Spearman Rho's Test

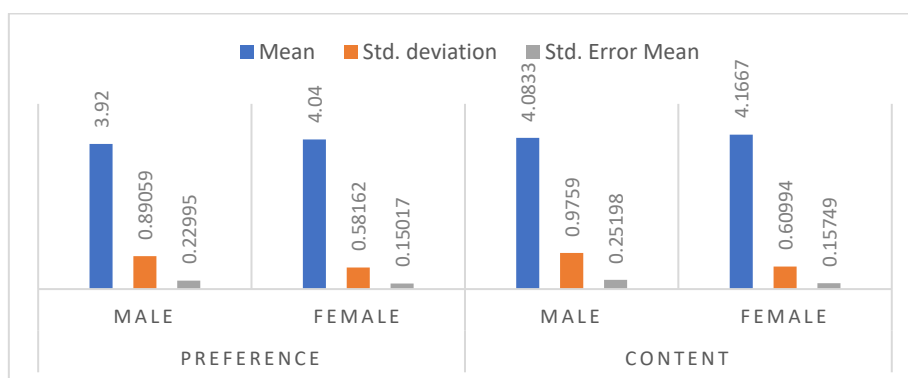
The correlation between these two categories, preference and content, can be calculated using Spearman's Rho. The result is as shown in Table 3.

**Table 3** Table of correlations between 2 categories 1) Preference and 2) Content, in addition to the independent T-test for 2 categories for male and female students.

|  |   |                         | Correlations                |                             |
|--|---|-------------------------|-----------------------------|-----------------------------|
|  |   |                         | Preference                  | Content                     |
| Spearman's rho   | Preference                                | Correlation Coefficient | 1.000                       | .937**                      |
|  |   | Sig. (2-tailed)         | .                           | <.001                       |
|  |   | N                       | 30                          | 30                          |
|  | Content                                   | Correlation Coefficient | .937**                      | 1.000                       |
|  |   | Sig. (2-tailed)         | <.001                       | .                           |
|  |   | N                       | 30                          | 30                          |
| **. Correlation is significant at the 0.01 level (2-tailed). |   |                         |                             |                             |
|  |   |                         | Preference                  | Content                     |
|  |   |                         | Equal variances not assumed | Equal variances not assumed |
| t-test for Equality of Means                                 | Significance                              | T                       | .437                        | .280                        |
|  |   | Df                      | 24.104                      | 23.490                      |
|  |   | One-Sided p             | .333                        | .391                        |
|  | Mean Difference                           | Two-Sided p             | .666                        | .782                        |
|  |   |                         | .12000                      | .08333                      |
|  | 95% Confidence Interval of the Difference | Std. Error Difference   | .27464                      | .29714                      |
|  |   | Lower                   | -.44670                     | -.53064                     |
|  |   | Upper                   | .68670                      | .69731                      |
|  |   |                         |                             |                             |

Table 3 shows the significant level for both preference and content is  $p < 0.001$  less than  $p = 0.01$  means that the correlation is significant. Based on the result, the Spearman's rho correlation coefficient is +0.937. The value is positive therefore result in preference and content are both positively correlated. In addition, the value of the correlation coefficient is larger than 0.5, which means it is strongly correlated.

## b) Dependent T-test



**Figure 2** The Means and Standard Deviation for Male and Female students based on the 2 categories

Referring to Figure 2 above, the mean preference score for male students is  $M=3.92$ ,  $SD=0.89$ , which is a little lower than the average preference score for female students,  $M=4.04$ ,  $SD=0.58$ . In contrast, both male and female scores for content are above 4.00, with the female score being higher ( $M=4.16$ ,  $SD=0.61$ ) than the male score ( $M=4.0833$  and  $4.1667$ ). Here, we can draw the conclusion that male students have higher standards to meet than female students do. For the content wise, both agree that the content is useful, informative, interactive and recommend to other students to use it. According to the independent t-test results in Table 2, there was no significant difference between male and female students for either category (preference:  $t=0.437$ ;  $p=0.666$ ; and content:  $t=0.280$ ;  $p=0.782$ ). Both categories have p-values greater than 0.05. The two null hypotheses for categories 1 and 2 are therefore retained. Which is there is no significant difference between marks for male and female students for preference as well as content.

## CONCLUSIONS

Various technology applications have been used to enhance learners' engagement and higher participation in lectures and other group learning sessions where collaborative and continuous efforts are required to identify or create the appropriate technology tools for efficient education based on educational theories (Tuma, 2021). The main goal of the study is to create new methods of learning, which is using the interactive calendar method. The measurement of effectiveness of this method has been determined. Male students find this thermal physics course to be less fascinating due to the mean score for interesting being less than 4 for female students, slightly larger than 4. As for the interactive calendar that has been introduced to the students, the survey was divided into two categories. Both preference and content are strongly correlated according to the Spearman Rho test. Which means, both categories are crucial in designing the interactive calendar. Besides that, an independent t-test shows that there is no significant difference between marks for male and female students for preference as well as content. Thus, hopefully these studies will help to develop a new learning environment in thermal physics course. Further research can be conducted with different Physics course. Education in research can help to close the bridge between Physics education and physics academicians (Nandiyanto et al., 2020). Additional demonstration experiments are required to clarify the theoretical background of the observed phenomena to increase the students' understanding (Klein et al., 2021). However, in this 21<sup>st</sup> century, the technological capabilities have exponential growth that will increase the complexity of task, and it is essential to clearly articulate the purpose of the education in the future (Grayson, 2020). Using contextually relevant media can also help bring science closer to students' realities, make learning more meaningful, and promote pedagogical strategies that are better aligned with their ways of thinking (Sony Yunior Erlangga et al., 2025). Using contextually relevant media can also help bring science closer to students' realities, make learning more meaningful, and promote pedagogical strategies that are better aligned with their ways of thinking (Erlangga et al., 2025).

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# DENSITY FUNCTIONAL THEORY INSIGHTS ON THE ELECTRONIC AND OPTICAL PROPERTIES OF ZnO IN SUNSCREEN APPLICATIONS

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**Abstract:** The structural, electrical, and optical properties of zinc oxide (ZnO) was performed using density functional theory using CASTEP software with exchange correlation of generalized gradient approximation (GGA) with Perdew-Burke-Ernzerhof (PBE). The band gap energy of ZnO was observed at 3.27 eV after a Hubbard U correction at energy. Incorporating these corrections brought the band gap energy closer to experimental values. The absorption shows that ZnO fall in the UV region compliment with the band gap energy obtain.

**Keywords:** Zinc oxide, Density functional theory, Electronic properties, Optical properties, Sunscreen

## INTRODUCTION

Zinc oxide (ZnO) is a versatile white powder widely applied across industries, with one of its most notable uses as an active ingredient in sunscreen, where it prevents the transmission of ultraviolet (UV) radiation through the skin (Kyu Bong Kim, 2017). As a physical sunscreen, ZnO acts as a broad-spectrum UV blocker and is generally considered less chemically reactive than organic UV filters. Although safer in many respects, some formulations may still cause skin irritation and pose environmental concerns (Desk, 2023). Beyond its UV-blocking function, ZnO exhibits anti-inflammatory and antimicrobial properties, making it particularly useful in sunscreens designed for sensitive or acne-prone skin. Its stability under diverse environmental conditions further strengthens its role as a reliable ingredient for daily sun protection products.

Generally, zinc oxide possesses a wide direct band gap of about 3.3–3.4 eV. These band gap energy value allow ZnO to absorb ultraviolet (UV) light effectively while maintaining good transparency in the visible spectrum, which is advantageous for applications in transparent electronics, UV photodetectors, and sunscreen formulations. ZnO most commonly crystallizes in the hexagonal wurtzite phase, which is thermodynamically stable under ambient conditions. ZnO has also attracted significant attention due to its abundance, low cost, non-toxicity, and chemical stability. Beyond traditional uses such as pigments, coatings, and cosmetics, it is now extensively studied for advanced applications including photocatalysis, gas sensing, solar cells, transparent conductive oxides, piezoelectric devices, and light-emitting diodes (LEDs).

Despite its widespread use, a deeper understanding of ZnO in term of band gap energy, absorption edge behaviour, and dielectric response is crucial for optimizing its role in cosmetic applications. Experimental studies provide valuable insights, but density functional theory (DFT) allows a more fundamental exploration of its properties at the atomic level. Standard DFT functionals often underestimate the band gap due to limitations in describing electron–electron interactions. Thus, incorporating Hubbard U corrections (DFT+U) or hybrid functionals becomes necessary for accurate band gap prediction and optical property analysis.

This study employs first-principles calculations within the CASTEP framework to investigate the structural, electronic, and optical properties of ZnO, with a particular focus on parameters relevant to sunscreen performance: the band gap, absorption edge, and dielectric function

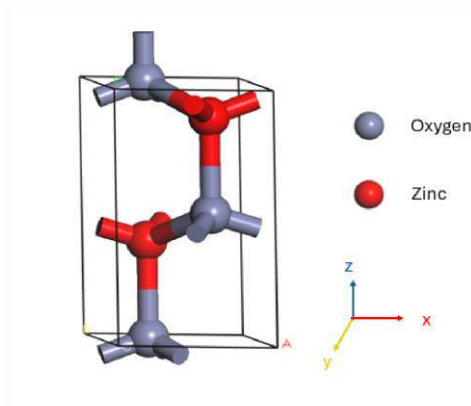
## METHODOLOGY

First-principles calculations based on plane-wave ultrasoft pseudopotentials method were performed for hexagonal ZnO using Cambridge Serial Total Energy Package (CASTEP) module in Materials Studio (MS) 8.0 developed by Accelrys Software Inc. The structural and electronic properties of ZnO were calculated using XC functionals from generalized gradient approximation GGA of Perdew-Burke-Ernzerhof (GGA-PBE). The valence electron configurations were treated as Zn-4s<sup>2</sup> 3d<sup>10</sup> and O-2s<sup>2</sup> 2p<sup>4</sup>. The optimized cut-off energy value for the plane-wave basis set of the electronic wave function was 340 eV. The integration in the Brillouin zone was performed using 5 × 5 × 4 k-points from the Monkhorst-Pack scheme. The geometrical optimization was performed using the total energy of 1.0 × 10<sup>-5</sup> eV/atom, the maximum force of 0.03 eV/Å, the maximum stress of 0.05 GPa and maximum

atomic displacement of  $1.0 \times 10^{-3}$  Å. The values of Hubbard U correction are  $U_d = 2$  eV and  $U_p = 10$  eV was referred to this paper since to optimized the Hubbard U energy required a long time (Hamzah, 2022)

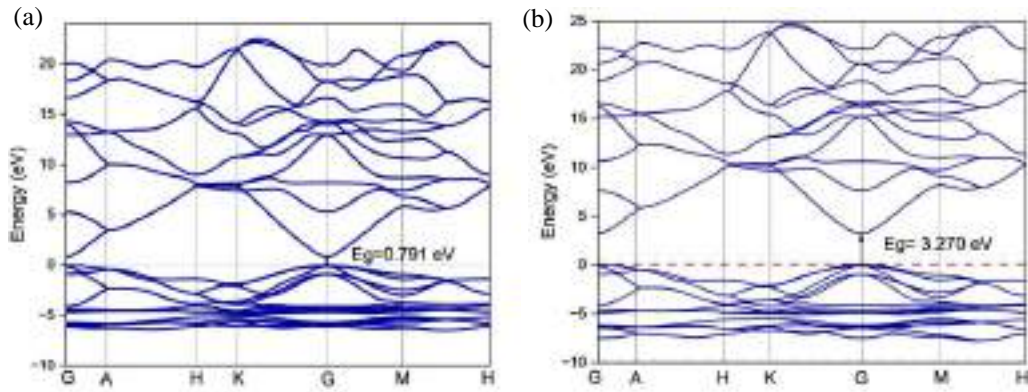
## FINDINGS

The hexagonal symmetry of ZnO plays a central role in determining its polar character, which underpins many of its physical properties, particularly its semiconducting and optoelectronic behaviour. The arrangement of atoms along the c-axis of the hexagonal lattice introduces polarity, which contributes to ZnO's wide direct band gap and its exceptional ability to absorb ultraviolet (UV) radiation. This characteristic makes ZnO highly effective in blocking a broad range of UV wavelengths, a property that is particularly relevant for sunscreen applications (Desk, 2023). Furthermore, the anisotropy of the wurtzite structure influences ZnO's optical responses, as the orientation of the c-axis governs the interaction of incident sunlight with the crystal lattice, thereby ensuring broad-spectrum UV protection. The optical transparency in the visible region, combined with strong UV absorption, makes ZnO one of the most desirable physical UV filters in cosmetic formulations. In addition to its optical advantages, the low-density yet robust HCP arrangement contributes to ZnO's notable mechanical and chemical stability, ensuring its durability under diverse environmental conditions. This structural stability, along with its non-toxic and non-reactive nature, reinforces ZnO's suitability for incorporation into daily-use cosmetic and skincare products, where long-lasting performance and safety are essential (Johnson & Lee, 2020). Figure 1 shows the crystal structure of unit cell of ZnO.

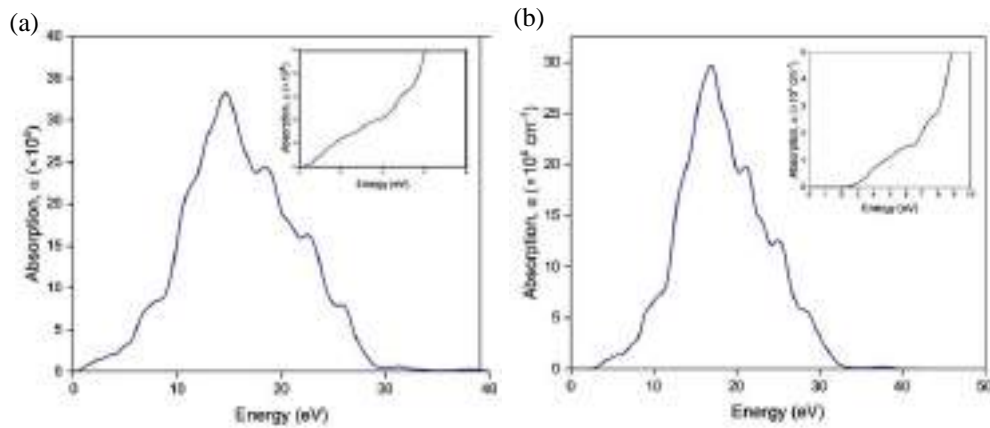


**Figure 1** Crystal structure of unit cell of ZnO

The band gap of a semiconductor is defined as the energy difference between the valence band maximum (VBM) and the conduction band minimum (CBM), representing the energy range where no electronic states exist. Figure 2(a) and Figure 2(b) shows the band structure of ZnO before and after Hubbard U correction. Using standard calculation, the band gap obtained is 0.791 eV which is underestimated by 77.27% from the experimental value of 3.48 eV. After the Hubbard U correction, it shows an improvement in band gap energy of 3.27 eV, in close agreement with experimental values (3.48 eV) (Huang & Lambrecht, 2014). The improvement is due to the correction of delocalized Zn 3d state by refining the electronic structure during the Hubbard U correction. The red dashed line at  $E = 0$  eV indicates the Fermi level, which in semiconductors lies within the band gap (Smijs & Pavel, 2011). The results confirm that ZnO is a direct band gap semiconductor, with both the VBM and CBM located at same Brillouin line. The corrected band gap of 3.27 eV corresponds to a photon wavelength of approximately 380 nm, within the UV-A (315–400 nm) and UV-B (280–315 nm) ranges (Smijs & Pavel, 2011). This allows ZnO to efficiently absorb harmful UV radiation while transmitting visible light ( $\lambda > 400$  nm;  $E < 3.1$  eV), explaining its effectiveness as a UV filter in sunscreens while maintaining transparency (Popov, Priezhev, & Lademann, 2005). In sunscreen formulations, photons with energies above 3.27 eV excite electrons from the valence band to the conduction band, thereby absorbing UV radiation and preventing skin damage. Meanwhile, visible light passes through unabsorbed, minimizing the whitening effect common in earlier products. This property underpins the widespread use of ZnO in commercial sunscreens such as CeraVe Hydrating Mineral Face Sunscreen (7% ZnO), Innisfree Daily UV Defense Mineral Sunscreen (17% ZnO), and Neutrogena Sheer Zinc Face Sunscreen (21.6% ZnO), where it provides broad-spectrum UV protection with cosmetic acceptability.

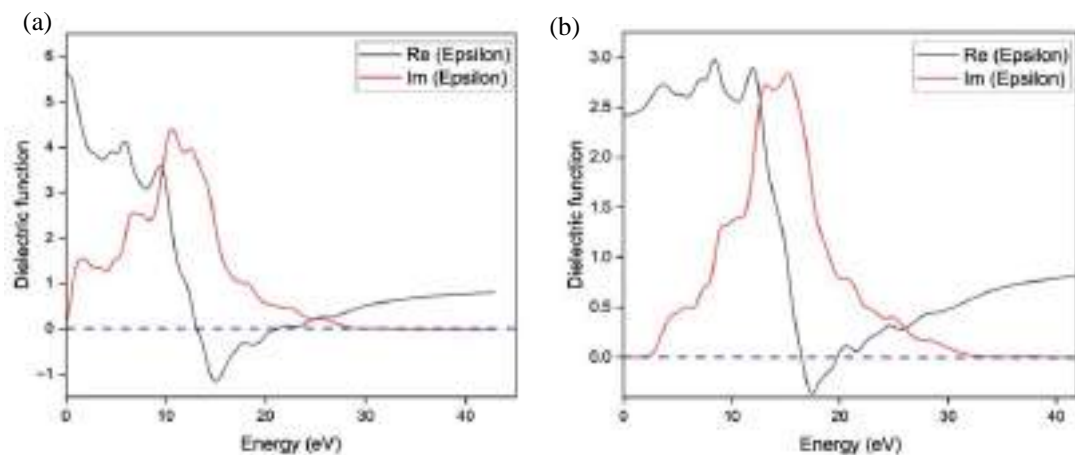


**Figure 2** (a) Band gap energy before Hubbard U correction (b) band gap energy after Hubbard U correction for ZnO



**Figure 3** (a) Absorption edge before Hubbard U correction (b) absorption edge after Hubbard U correction for ZnO

Figure 3(a) and Figure 3(b) shows the absorption edge of ZnO before and after Hubbard U correction. It is observed that the improvement of absorption edge is significantly with the data obtained in the band structure. From this figure, the absorption edges fall in between  $\sim 3.1$  eV which shows a good agreement with the band structure in previous discussion was shown in Figure 3(b). The absorption edge of 3.1 eV falls in UV spectrum which shows that this material has capability to absorb the UV light effectively. From this data, it is significant with most of the sunscreen or cosmetic product that use ZnO as one of their ingredients.



**Figure 4** (a) Dielectric function before Hubbard U correction (b) dielectric function after Hubbard U correction for ZnO

Figure 4(a) and 4(b) shows the dielectric function of ZnO before and after Hubbard U correction. It clearly seen that there is an improvement in dielectric after Hubbard U correction. The dielectric function can be split into two parts: the imaginary part,  $\text{Im}(\epsilon)$ , represented by the red line, and the real part,  $\text{Re}(\epsilon)$ , represented by the black line. The imaginary part describes how much energy is dissipated in the material by the absorption of light, the real part describes how the ZnO plant affects the speed of propagation of electromagnetic waves due to a change in the refractive index that changes the light dispersion (Smith et al., 2023). The real part of the dielectric function,  $\text{Re}(\epsilon)$ , shows a static dielectric constant of approximately 2.5–3 at low photon energies, which gradually decreases and becomes negative in the range of 15–20 eV, indicating the occurrence of plasmon resonance. Beyond this energy,  $\text{Re}(\epsilon)$  stabilizes toward positive values, suggesting weaker dispersion effects at higher energies. The imaginary part,  $\text{Im}(\epsilon)$ , reveals the optical absorption edge at ~3.2 eV, in agreement with the corrected band gap of 3.27 eV. A strong absorption peak is observed around 12–14 eV, attributed to interband transitions from O 2p to Zn 4s/4p states, followed by a gradual decline in absorption beyond 20 eV. The onset of absorption within the ultraviolet region confirms that ZnO is a direct band gap semiconductor capable of efficiently blocking UV-A and UV-B radiation, while its transparency to visible light ( $\lambda > 400$  nm) ensures its cosmetic appeal in sunscreen formulations. These results demonstrate that DFT+U provides an accurate description of ZnO's optical behaviour, thereby reinforcing its role as a safe and effective broad-spectrum UV filter.

## CONCLUSIONS

In this work, the electronic, and optical properties of ZnO were investigated using density functional theory with Hubbard U correction. The corrected band gap of 3.27 eV aligns closely with experimental values, confirming ZnO as a wide direct band gap semiconductor. The corresponding absorption edge at ~3.0–3.2 eV indicates strong absorption in the UV-A and UV-B regions while maintaining transparency in the visible spectrum. Analysis of the dielectric function further supports this behaviour, showing a static dielectric constant of 2.5–3 and pronounced absorption peaks arising from interband transitions. These findings emphasize ZnO's dual advantage as an efficient UV-blocking material and a transparent component in sunscreen formulations, underscoring its potential for safer and more effective cosmetic applications.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# CYTOTOXIC EVALUATION OF 6-METHOXY TETRAHYDRO- $\beta$ -CARBOLINE DERIVATIVES (6MTH $\beta$ C) IN HUMAN NEUROBLASTOMA (SK-N-SH) CELLS

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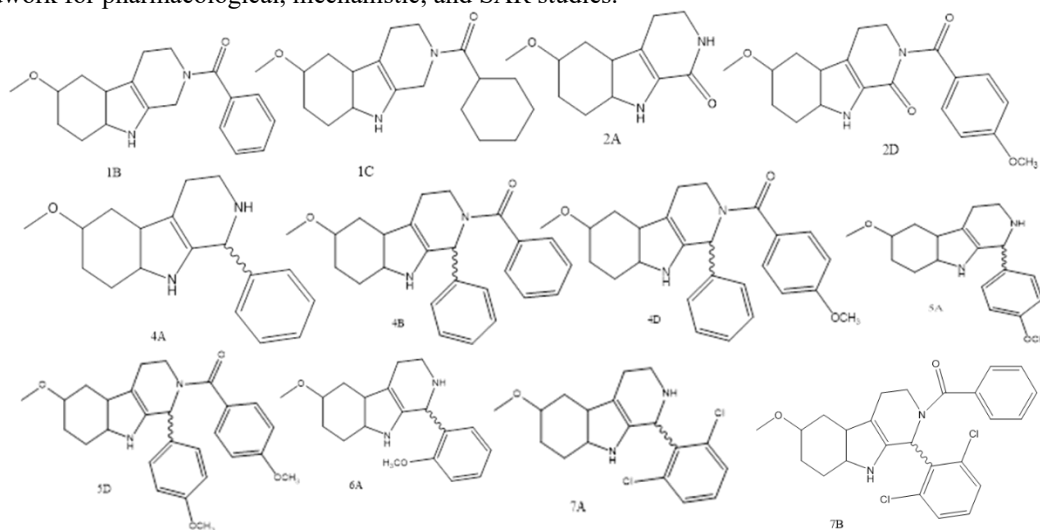
**Abstract:** Tetrahydro- $\beta$ -carboline (TH $\beta$ C) alkaloids, a varied group of indole-derived chemicals, demonstrate pharmacological actions including anticancer, antibacterial, antioxidant, and neuroactive effects in nature. In this study, 6-methoxy-tetrahydro- $\beta$ -carboline (6MTH $\beta$ C) has been synthesized and evaluated for its toxicity against the human neuroblastoma cell line (SK-N-SH) using the MTT assay. The assay was conducted at a concentration of 50  $\mu$ M of each compound. The findings showed that four compounds exhibited toxicity toward SK-N-SH cells, while eight compounds demonstrated more than 50% cell viability. This study provides fundamental toxicological insights into these compounds and lays the groundwork for pharmacological and potential new treatment possibilities related to neurodegenerative disease.

**Keywords:** Cytotoxicity, SK-N-SH, Tetrahydro- $\beta$ -carboline, Human neuroblastoma cell

## INTRODUCTION

Tetrahydro- $\beta$ -carboline (TH $\beta$ C) alkaloids, a diverse class of indole-based compounds, exhibit pharmacological activities such as anticancer, antimicrobial, antioxidant, and neuroactive properties throughout nature (Herraiz & Guillén, 2018; Cao et al., 2007). Wang et al. (2021) found that structural modifications to the TH $\beta$ C scaffold, particularly substitutions at critical locations, enhance bioactivity and selectivity. Methoxy-substituted derivatives may interact with biomolecular targets of cancer therapy, making them interesting. The varied clinical behaviour and resistance to traditional treatments make neuroblastoma, one of the most frequent extracranial solid tumours in children, a serious therapeutic challenge (AlKhazal et al., 2025). The SK-N-SH human neuroblastoma cell line is commonly used to test the cytotoxic and neuropharmacological effects of synthetic and natural chemicals *in vitro* (Zhou et al., 2021). Thus, identifying novel small compounds that induce cytotoxicity in neuroblastoma cells is crucial for discovering new treatment options.

Introducing a methoxy group to 6-methoxy-tetrahydro- $\beta$ -carboline (6MTH $\beta$ C) derivatives (Figure 1) may alter their electrical characteristics, lipophilicity, and receptor-binding affinity, thereby affecting their lethal potential. Despite their well-documented bioactivities, limited research exists on methoxy-substituted analogues of TH $\beta$ C alkaloids against neuroblastoma. In this work, 6MTH $\beta$ C derivatives will be tested for cytotoxicity *in vitro* utilising the SK-N-SH cell line. This study provides fundamental toxicological insights into these compounds and lays the groundwork for pharmacological, mechanistic, and SAR studies.



**Figure 1** Structure of 6-methoxy-tetrahydro- $\beta$ -carboline (6MTH $\beta$ C) derivative

## METHODOLOGY

The toxicity study of twelve 6MTH $\beta$ C compounds was carried out using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, as described by Mosmann (1983). The cell culture experiments were performed using aseptic techniques in a sterile biosafety cabinet. The human neuroblastoma cell line (SK-N-SH) was cultured in Eagle's Minimum Essential Medium, EMEM (ATCC 30-2003), supplemented with 10% heat-inactivated fetal bovine serum and 1% of 10,000 U/ml penicillin and 10 mg/mL streptomycin per mL. Other solutions, such as phosphate buffer saline (PBS) and distilled water, were autoclaved before use.

The cells were grown as a monolayer at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>. Cells with 80% confluency were harvested from a 75 cm<sup>2</sup> culture flask. The cells were rinsed with 10 mL of PBS, trypsinized using 5 mL of Trypsin-EDTA 1x, diluted in growth medium, and then centrifuged at 800 g. Cells were then counted using a hemocytometer and plated at a density of  $2 \times 10^4$  cells per well in a 96-well plate. The cells were incubated for 24 hrs at 37 °C to confluency, with 5% CO<sub>2</sub> in a humidified atmosphere. All the 6MTH $\beta$ C compounds were dissolved in 100% DMSO (stock solution) at 20 mM concentration. For the working solution, 5  $\mu$ L of stock solution was diluted with fresh growth medium to 500  $\mu$ L.

100  $\mu$ L of a working solution containing compounds in triplicate at a final concentration of 50  $\mu$ M in a medium was added to the cells and incubated for another 48 hrs. As a negative control, cells were treated with 200  $\mu$ L of the vehicle in growth media. Cisplatin was used as a positive control drug. The final concentration of DMSO was kept at 0.5 % v/v in this experiment. After 48 hrs, 20  $\mu$ L of aqueous MTT solution (5 mg/mL in PBS) was added to each well, and the plate was covered with aluminum foil and incubated at 37 °C for 3 hrs in a CO<sub>2</sub> incubator in the dark. After 3 hrs, the MTT solution was carefully decanted off, and dark blue formazan crystals were extracted from the cells with 100  $\mu$ L of DMSO in each well. The optical density (OD) was measured with a 96-well ELISA microplate reader at 570 nm, with the reference filter set to 630 nm. All MTT assays were repeated three times. The cell viability of the tested compounds was estimated by directly comparing the OD value of the treated cells with that of the untreated cells. Percentage cell viability was calculated using the formula (Kyung et al., 2005):

$$\% \text{ Cell Viability} = \frac{OD \text{ treated cell} - \text{blank}}{OD \text{ untreated cell} - \text{blank}} \times 100$$

## FINDINGS

Twelve compounds were successfully screened for toxicity using the MTT assay. The reduction of the MTT assay was conducted to assess the cell viability. MTT is a water-soluble tetrazolium salt that is reduced to a coloured water-insoluble salt by metabolically viable cells. The number of living cells present is proportional to the transformed atoms of MTT tetrazolium salt into the blue-coloured product. The percentage of cell viability for each compound is summarized in Table 1. The MTT assay was conducted for twelve 6MTH $\beta$ Cs derivatives on SK-N-SH at a concentration of 50  $\mu$ M. The positive control of the experiment is cisplatin. The results indicated that the eight 6MTH $\beta$ Cs derivatives showed more than 50% cell viability. Four compounds (2D, 4B, 4D, 5D) showed a percentage cell viability of less than 50% and are considered toxic at a concentration of 50  $\mu$ M, as suggested by the previous report (Akundi et al., 2004).

**Table 1** Percentage of cell viability for each compound

| Compound  | % Cell viability  |
|-----------|-------------------|
| Cisplatin | 1.67 $\pm$ 0.48   |
| 1B        | 92.44 $\pm$ 3.12  |
| 1C        | 62.20 $\pm$ 2.21  |
| 2A        | 101.21 $\pm$ 4.05 |
| 2D        | 16.75 $\pm$ 1.74  |
| 4A        | 92.62 $\pm$ 2.18  |
| 4B        | 39.91 $\pm$ 4.66  |
| 4D        | 49.01 $\pm$ 7.62  |
| 5A        | 100.97 $\pm$ 1.59 |
| 5D        | 50.90 $\pm$ 0.63  |
| 6A        | 72.54 $\pm$ 4.11  |
| 7A        | 91.26 $\pm$ 0.53  |
| 7B        | 80.97 $\pm$ 0.23  |

Cell viability and/or cell proliferation are good indicators of cell health. Physical and chemical agents can influence cell health and metabolism. Cell toxicity can be caused by various processes, including cell membrane disintegration, protein synthesis suppression, irreversible binding to receptors, inhibition of polydeoxynucleotide

elongation, and enzymatic reactions (Aslantürk, 2018). In this study, in vitro toxicity against SK-N-SH was conducted to screen the 6MTH $\beta$ Cs derivatives. Generally, all the 6MTH $\beta$ Cs derivatives have the same skeleton (parent) but different substituents at C1 and N2. Using an indicator reported by Akundi and coworkers (2004), eight compounds (1B, 1C, 2A, 4A, 5A, 6A, 7A, and 7B) were considered nontoxic, as they had a percentage cell viability of more than 50%. At the same time, four compounds, namely 2D, 4B, 4D, and 5D, were toxic as their percentage of cell viability was less than 50%. The results clearly showed that different substituents at the C1 and N1 affect the properties of the compounds. From the results, it is evident that the compounds with substituents B (phenyl) and D (methoxyphenyl) exhibit toxicity effects on the SK-N-SH cells. The 2D was the most toxic to the cells since cell viability was the lowest (17%).

However, for the same series of compounds, such as 4B and 4D, the 4B compound is more toxic, possibly due to the absence of the methoxy group at the benzene ring attached to the N2. In contrast, the 4A compound with a hydrogen atom substituent at position N2 was safe. Compared across the series, but with similar substitutions in C1, such as between 1B and 4B, only 4B exhibits toxic properties due to the phenyl substituent attached to the C1 atom. When comparing 2D, 4D, and 5D, 2D was the most toxic compound, suggesting that the presence of a carbonyl group at the C1 position caused the toxic effect. This toxicity study involved only a limited number of compounds; therefore, a larger number of compounds are required to produce meaningful information. The previous report showed that tetrahydro- $\beta$ -carbolines and 1,3,5-triazine hybrids are anticancer agents against eight human cancer cell lines and the normal human fibroblast (NIH3T3) cell line (Kumar et al., 2010). Other studies have reported that TH $\beta$ Cs derivatives have toxicity in both in vitro and in vivo models, including neurodegeneration and Parkinson-type symptoms (Akundi et al., 2004). Thus, there is a possibility that the 6MTH $\beta$ Cs derivatives may exhibit toxic effects against specific cell lines.

## CONCLUSIONS

The current research revealed that 6-methoxy-tetrahydro- $\beta$ -carboline (6MTH $\beta$ C) derivatives display significant cytotoxic effects on the SK-N-SH human neuroblastoma cell line. These findings underscore the promise of methoxy-substituted TH $\beta$ C scaffolds as viable options for advancing anticancer research. The observed cytotoxicity offers initial indications of biological activity; nevertheless, further research is necessary to clarify the mechanisms of action, assess selectivity for cancer cells compared to normal cells, and investigate structure–activity correlations. This research establishes a basis for progressing 6MTH $\beta$ C derivatives into extensive pharmacological and toxicological studies.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# HONEY SOLUTION AS A NATURAL BIOSTIMULANT FOR THE GROWTH OF *Capsicum annuum* L.

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**Abstract:** Honey is a natural product rich in sugars, amino acids, vitamins, and bioactive compounds, with potential applications as a sustainable plant biostimulant. This study evaluated the effects of different concentrations of honey solution on the growth of *Capsicum annuum* by measuring plant height over a five-week period. Five treatments were prepared: fertilizer only (control), and honey solutions at concentrations of 5%, 10%, 15%, and 20% (v/v) without fertilizer. Each treatment was replicated four times, and solutions were applied fortnightly at weeks 1, 3, and 5. Plant height was recorded weekly, and mean values were compared across treatments. The results showed that the 20% honey solution produced the greatest increase in average plant height ( $11.33 \pm 1.15$  cm), whereas the 5% solution resulted in the lowest growth ( $1.63 \pm 0.48$  cm). The control achieved  $6.75 \pm 0.96$  cm, which was slightly higher than the 10% ( $6.00 \pm 0.82$  cm) and 15% ( $6.50 \pm 1.29$  cm) treatments. These findings indicate that honey supplementation influenced growth in a concentration-dependent manner, with only the higher concentration producing substantial improvement compared to fertilizer alone. The stimulatory effects of honey are likely linked to its nutrient content and bioactive compounds, which may enhance microbial activity, nutrient uptake, and plant metabolic processes. In conclusion, honey solution particularly at 20% concentration demonstrated potential as an eco-friendly biostimulant for enhancing the growth of *C. annuum*. Future research should investigate additional growth parameters, such as leaf development, biomass accumulation, and fruit yield, and extend testing to other crops and environmental conditions. These findings support the potential of honey as a sustainable alternative to synthetic fertilizers in horticultural production.

**Keywords:** *Capsicum annuum*, Honey solution, Plant growth, Natural biostimulant, Sustainable agriculture

## INTRODUCTION

*Capsicum annuum* is one of the most widely cultivated vegetable crops, valued both for its nutritional content and economic importance (Bosland & Votava, 2012; Taiz et al., 2018). Growth and productivity of *C. annuum* are significantly influenced by soil fertility and nutrient availability. Traditionally, chemical fertilizers are applied to enhance crop growth, however, excessive use of such fertilizers has led to environmental concerns and soil degradation (Savci, 2012). This has increased the demand for eco-friendly and sustainable alternatives, such as plant biostimulants, organic amendments, and natural growth enhancers (Du Jardin, 2015; Feitosa de Vasconcelos & Garófalo Chaves, 2019; Peña-Calzada et al., 2025).

Honey, a natural product rich in sugars, amino acids, vitamins, enzymes, and bioactive compounds, has been recognized not only for its nutritional value but also for its potential use in agriculture (Bogdanov et al., 2008; Ahmed et al., 2007). Studies have shown that honey application can enhance plant growth, yield, and biochemical properties by improving phytohormone balance, antioxidant activity, and nutrient uptake (Belal et al., 2023; Moussa et al., 2024; Sadou et al., 2025). In particular, honey has been reported to alleviate stress conditions and stimulate physiological functions similar to other biostimulants (Cattivello, 2019; Henderson et al., 2025).

Therefore, the present study aims to evaluate the effects of honey solution at different concentrations (5, 10, 15, and 20% v/v) on the growth of *C. annuum*. By comparing honey treatments with fertilizer controls, this research explores the potential of honey as an eco-friendly biostimulant for sustainable agriculture.

## METHODOLOGY

### Plant material

The research was conducted using *C. annuum* plants (20–50 cm in height), cultivated in polythene bags filled with nursery soil. The experiment was carried out at Universiti Teknologi MARA (UiTM) Perak Branch, Tapah Campus, Perak, Malaysia.

## Experimental design

The study was arranged in a completely randomized design with five treatments and four replications each. The treatments consisted of:

- **Treatment A (Control):** Fertilizer solution
- **Treatment B:** 5% (v/v) honey solution
- **Treatment C:** 10% (v/v) honey solution
- **Treatment D:** 15% (v/v) honey solution
- **Treatment E:** 20% (v/v) honey solution

## Preparation of solutions

**Fertilizer solution:** 10 g of fertilizer was weighed and dissolved in distilled water in a 100 mL beaker. The solution was transferred into a 100 mL volumetric flask and diluted to the mark with distilled water. The flask was inverted several times to ensure homogeneity.

**Honey solutions:** Different concentrations of honey solutions were prepared separately in 100 mL volumetric flasks. For each solution, the required volume of honey (5 mL, 10 mL, 15 mL, and 20 mL for 5%, 10%, 15%, and 20% v/v solutions, respectively) was measured and transferred into the flask. Distilled water was added to make up to 100 mL final volume, and the flask was gently swirled until the honey was completely dissolved.

## Application of treatments

Plants were treated with the respective solutions once every two weeks (week 1, week 3, and week 5).

## Data collection

The growth parameter measured in this study was plant height. Measurements were recorded weekly for all treatments throughout the five-week experimental period.

## FINDINGS

### Plant growth performance

The effect of different honey concentrations on the growth of *C. annuum* was evaluated by measuring plant height over a five-week period. The results (Table 1) showed that the 20% honey solution (Treatment E) produced the highest average plant height (11.33±1.15 cm), while the 5% treatment (Treatment B) resulted in the lowest growth (1.63±0.48 cm). The control (6.75±0.96 cm) performed slightly better than the 10% (6.00±0.82 cm) and 15% (6.50±1.29 cm) treatments, indicating that honey supplementation influenced plant growth in a concentration-dependent manner.

**Table 1.** Average height of *Capsicum annuum* under different treatments

| Treatment | Description               | Average height (cm) |
|-----------|---------------------------|---------------------|
| A         | Fertilizer only (Control) | 6.75±0.96           |
| B         | 5% honey solution         | 1.63±0.48           |
| C         | 10% honey solution        | 6.00±0.82           |
| D         | 15% honey solution        | 6.50±1.29           |
| E         | 20% honey solution        | 11.33±1.15          |

The superior growth observed in the 20% honey treatment suggests that higher concentrations may provide beneficial levels of sugars, amino acids, and micronutrients, thereby stimulating metabolic activity and nutrient uptake (Bogdanov et al., 2008). Honey also enhances microbial activity in the rhizosphere, which could further improve nutrient availability. In contrast, the poor growth at 5% may reflect insufficient nutrient contribution or potential osmotic effects that limited water uptake. This aligns with Du Jardin (2015), who emphasized that the efficacy of biostimulants is strongly dosage-dependent.

These findings are consistent with previous studies reporting that honey can act as a natural growth enhancer across plant species. Positive growth responses were recorded in beans (Belal et al., 2023), sage (*Salvia officinalis*) (Moussa et al., 2024), and basil (*Ocimum basilicum*) (Sadou et al., 2025), suggesting a broad-spectrum effect. The stimulatory impact is likely linked to honey's bioactive compounds, which may influence phytohormone regulation, enzyme activity, and photosynthetic performance (Ahmed et al., 2007; Henderson et al., 2025; Feitosa de Vasconcelos & Garófalo Chaves, 2019).

Importantly, honey supplementation provides an eco-friendly alternative to synthetic fertilizers, which are often associated with soil degradation and environmental pollution (Savci, 2012). The observed growth improvements support the potential role of honey in sustainable agriculture, where natural inputs are prioritized to maintain productivity while reducing ecological impact (Peña-Calzada et al., 2025).

Overall, the results indicate that honey, particularly at higher concentrations, can act as an effective natural biostimulant for *C. annuum*. However, further studies are needed to assess additional growth parameters, including leaf number, biomass accumulation, and fruit yield, as well as long-term impacts on soil fertility and microbial dynamics.

## CONCLUSIONS

This study demonstrated that honey solution has potential as a natural biostimulant for the growth of *C. annuum*. Among the treatments tested, the application of 20% honey solution resulted in the greatest increase in plant height ( $11.33 \pm 1.15$  cm), outperforming both the control and lower concentrations. In contrast, the 5% honey treatment produced the lowest growth ( $1.63 \pm 0.48$  cm), suggesting that concentration plays a critical role in determining the effectiveness of honey as a growth enhancer. The results indicate that honey at higher concentrations may provide essential nutrients and bioactive compounds that stimulate plant growth, while lower concentrations may be less effective. These findings support the potential use of honey as an eco-friendly supplement to chemical fertilizers in sustainable agriculture. For future research, it is recommended to evaluate additional growth parameters, including leaf development, biomass accumulation, and fruit yield, as well as to investigate the effects of honey solution across different plant species and under varying environmental conditions, particularly its long-term impact on soil fertility and microbial activity.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# A PRELIMINARY STUDY OF ROSELLE EXTRACT AS ANTIBACTERIAL TOWARDS *Staphylococcus epidermidis* BY DISK DIFFUSION METHOD

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**Abstract:** Roselle also known as *Hibiscus sabdariffa*, calyx extracts are frequently used in traditional folk medicine and could be useful in natural sources of antimicrobial activity. The calyces are developed with substances that have rich content of antimicrobial properties, antioxidants, and anthocyanins. This research aims to investigate the antibacterial properties of roselle extract towards *Staphylococcus epidermidis* and record inhibition zone produced by roselle extract towards *S. epidermidis*. This involved the disk diffusion method whereby sterile paper disks with various concentrations of roselle extract were inserted onto an agar plate strewn with *S. epidermidis*, and the inhibition zone was measured after incubating. Results showed that *H. sabdariffa* induced a significant zone of inhibition of 12 mm for roselle extract A and 11 mm roselle extract B against which is a duplicated sample against *S. epidermidis* in the disk diffusion method. Since this bacterium is a common cause of skin infections, the potential of roselle would be most beneficial in developing a skin preparation that would target bacterial growth with no harsh chemicals. This study has led to further investigations into the antimicrobial mechanisms of roselle since it could also be more beneficial in understanding how it interacts with bacterial cells for better clinical applications. The other way could be testing its effectiveness in the real medical and skin care environment, which would end in safer and more efficient natural products.

**Keywords:** Antimicrobial activity, Disk diffusion, *Hibiscus sabdariffa*, *Staphylococcus epidermidis*

## INTRODUCTION

Throughout human history, natural products and traditional remedies have been essential to the development of medicine, offering a foundation for therapeutic practices long before the emergence of modern pharmaceuticals. From the earliest civilizations to contemporary times, humans have relied on the abundant resources provided by nature to address ailments and promote well-being. These natural remedies, often derived from plants, minerals, and other organic sources, are rich in bioactive compounds with diverse medicinal properties. One such example is the *H. sabdariffa*, which is also commonly known as roselle. According to Da-Costa-Rocha et al., 2014, roselle is known to be rich in bioactive compounds, such as anthocyanins and other flavonoids, organic acids, and polysaccharides, which are responsible for its antioxidant, antibacterial, anti-inflammatory, hepatoprotective and anti-cholesterol activities.

In recent years, scientists and researchers have extensively explored the potential of natural products as alternatives to synthetic antibiotics, driven by the growing concern over the emergence of antibiotic-resistant bacteria. This recently evolved characteristic of pathogens, in which they develop resistance to conventional antibiotics, poses a significant threat to global health. Among those bacteria, *S. epidermidis* is noteworthy, as it is commonly found on human skin, but can also act as an opportunistic pathogen. They are particularly concerned due to their ability to produce biofilms, a characteristic that enables these bacteria to thrive as a commensal organism, as well as its resistance to antibiotics. Biofilm formation enables this organism to evade the host immune system (Kleinschmidt, S. L., 2018). This further highlights the urgent need for effective, natural antibacterial agents, such as roselle, to combat these resistant pathogens. To achieve this, this study employs standard microbiological techniques, which are the disc diffusion method and the serial dilution method. In the disc diffusion method, small disks impregnated with roselle extract will be placed onto a plate of agar-infused bacteria to determine and measure the inhibition zone. The inhibition zone, in this regard, is an area of no bacterial growth. The larger the inhibition zone, the more effective the roselle extract is against bacteria. Preliminary results indicate that roselle extract exhibits significant inhibitory effects on *S. epidermidis*, particularly at higher concentrations.

The emergence of antibiotic resistance among bacterial pathogens poses a critical threat to global health. *S. epidermidis*, a species of bacteria that were once considered relatively harmless, has become a significant contributor to hospital-acquired infections, particularly with catheterized patients or those who had undergone orthopaedic or breast implants (Oliveira et al., 2018). Its ability to form biofilms further complicates treatment, as biofilms protect the bacteria from both the immune system as well as antibiotics. Despite its long history of

medicinal use, there is still limited scientific evidence supporting the antibacterial potential of roselle extract against *S. epidermidis*. While previous studies have demonstrated the antimicrobial activity of roselle against other bacterial strains, its specific effect against this opportunistic pathogen remains unclear. Addressing this knowledge gap is crucial, as it could pave the way for developing plant-based antimicrobial solutions that are not only effective and sustainable but also beneficial for current and future generations. This study is significant in addressing the pressing need for an alternative solution to combat antibiotic-resistant bacteria such as *S. epidermidis*. By evaluating and studying the antibacterial effects of roselle juice on this species using the disc diffusion technique and the serial dilution method, the research contributes to a growing body of knowledge on plant-based antimicrobial agents. Roselle extract, being a natural and widely accessible product typically found in tropical and subtropical regions, including China, Egypt, Indonesia, Mexico, Nigeria, Thailand, and Saudi Arabia (Izquierdo-Vega et al., 2020), has the potential to serve as an eco-friendly and cost-effective alternative to synthetic antibiotics. Furthermore, the findings of this study may have a broader implication for various industries. For instance, if proven effective, roselle extract or the flower itself could be utilised into therapeutic applications, integrated into pharmaceutical products, or even incorporated into the food industry (Remigio et al., 2024). Furthermore, this research could also inspire further studies on the bioactive compounds in roselle and their specific mechanism of action, which ultimately could contribute to the global fight against antibiotic-resistant bacteria.

## **METHODOLOGY**

### **Nutrient Agar Medium Preparation**

A 14.4g of agar powder was weighed and added to a 500 mL scotch bottle. A 500 mL of distilled water was added and shaken well until all the agar powder was dissolved. The bottle was then closed, ensuring the cap was not tightly sealed to allow for autoclaving. The scotch bottle was placed in the autoclave for 15 minutes at 121°C under 15 lbs of pressure. A proper workplace was prepared by sterilizing all surface of bench or in laminar airflow cabinet. Once the pressure of the autoclave reached 0, the scotch bottle was removed and allowed to cool briefly to prevent the agar from solidifying slowly. The agar was carefully poured into the petri dishes under a laminar airflow cabinet to minimize the risk of contamination. Each plate was filled with nutrient agar, ensuring its surface was evenly covered. Agar petri was left under the UV light of laminar airflow cabinet for 30 minutes, after that the Petri dishes were sealed, flipped upside down and stored in a chiller for the next experiment.

### **Nutrient Broth Medium Preparation**

A 2.6 g of nutrient broth powder was weighed and added to a scotch bottle. 200 mL of distilled water was added to the bottle and shaken well to dissolve the powder. The bottle was then closed, ensuring the cap was not tightly sealed to allow for autoclaving. The scotch bottle containing the nutrient broth medium and universal bottles was autoclaved for 15 minutes at 121°C under 15 lbs of pressure. After autoclaving, all equipment was removed and allowed to cool for half an hour. The broth was then poured into three universal bottles under sterilized conditions to prevent contamination. The universal bottles were subsequently stored in a chiller for future use.

### **Roselle Extract Preparation**

The roselle extract was prepared using roselle powder. The 4.1g of powder was weighed using a weighing balance and transferred into a clean beaker. A 4 mL of sterilized distilled water was then added to the beaker. The mixture was stirred using a clean glass rod until the roselle powder was completely dissolved. Any excess solution was poured into a sterilized scotch bottle for storage or further use. For this research a duplicated sample of roselle have been used (roselle extract A and roselle extract B).

### **Disk Diffusion Method**

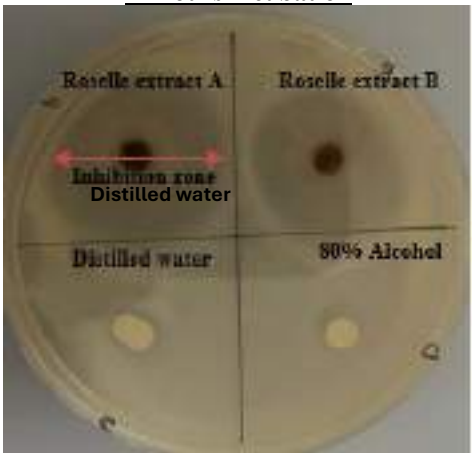
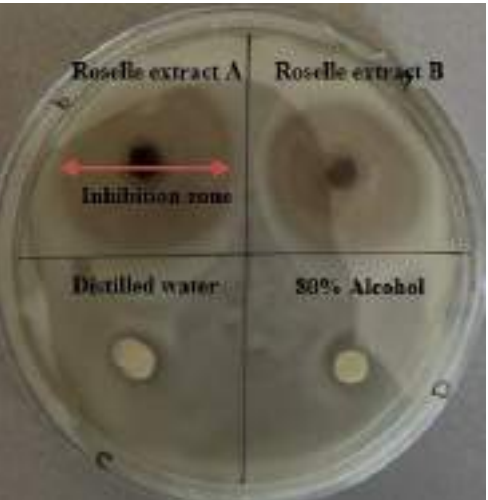
Four sections were drawn on the back of the agar petri dish using a marker pen, labelled as follows: A duplicates sample of roselle extract (roselle extract A and roselle extract B), C for distilled water (negative control) alcohol (positive control), and D for alcohol (positive control). A 200 µL volume of *S. epidermidis* bacteria was micropipette and placed in the centre of the agar. The cell spreader was soaked in alcohol, flamed using a Bunsen burner, allowed to cool, and then used to spread the bacteria across the agar surface (Louis, S. 2018). This process was repeated 2 times with new petri dishes. Filter paper was punched using a paper punch to create disks and autoclaved it. Three layers of the punched disk paper that was already autoclaved were stacked, and 100 µL of roselle extract was micropipette onto the stacked disks. Using forceps, the disk was placed in the agar centre of sections A and B. This method was repeated for alcohol and distilled water. After preparing all three petri dishes, they were incubated for 24 hours, and the inhibition zones were observed and measured over the following 2 to 3 days (Louis, S. 2018).

## FINDINGS

### Antibacterial Activity of *Hibiscus sabdariffa* Extract Against *Staphylococcus epidermidis*

This study investigated the antibacterial effects of *Hibiscus sabdariffa* extract on *Staphylococcus epidermidis* using disk diffusion methods. An observation took 48 hours to record the presence of inhibition zone. The disk diffusion test revealed inhibition zones of roselle extract A was 10mm at the first 24 hours of incubation. The inhibition zone increases to 12 mm after 48 hours of incubation period. The results of antimicrobial activity of roselle extract B showed 9mm at the first 24 hours and 11mm after 48 hours of incubation. This demonstrated significant antibacterial activity, whereas alcohol (positive control) produced smaller inhibition zones of 2-3 mm. Distilled water (negative control) unexpectedly showed a 1 mm inhibition zone, suggesting possible contamination or experimental error. The roselle extract exhibits greater antibacterial activity. These findings align with Jabeur et al., 2017, who attributed the antibacterial properties of *H. sabdariffa* to bioactive compounds such as anthocyanins and flavonoids. Previous studies by Cid-Ortega & Guerrero in the year 2015, have also demonstrated the antimicrobial potential of *H. sabdariffa* against *Staphylococcus aureus* and *Escherichia coli*. These results further support by Ventola (2015), highlighting the promise of natural antimicrobials in addressing antibiotic resistance. In conclusion, *H. sabdariffa* extract exhibited significant antibacterial activity against *S. epidermidis*, reinforcing its potential as a natural alternative to synthetic antimicrobial agents.

**Table 1.** Diameter Zone of Inhibition at 24 and 48 hours of inhibition

| Incubation Period   | Diameter zone of inhibition (mm) |                   |                               |                       |
|---|----------------------------------|-------------------|-------------------------------|-----------------------|
|   | Roselle extract A                | Roselle extract B | Distilled water (+ve control) | Alcohol (-ve control) |
| <p><b>24 hours incubation</b></p>   | 10                               | 9                 | 1                             | 2                     |
| <p><b>48 hours incubation</b></p>  | 12                               | 11                | 2                             | 3                     |

## CONCLUSION

This preliminary study confirmed that the roselle extract has significant antibacterial properties against *S. epidermidis* due to the presence of the inhibition zone as it possessed the ability to inhibit the bacterial growth. These findings posited that the extract is rich in bioactive compounds that exhibit antimicrobial properties, hence promising a source of natural antibacterial applications. This research complements the rising interest in plant-based alternatives to pharmaceutical antibiotics, especially for resistance against pathogens like *S. epidermidis*, or even incorporated into the food industry. If further studies confirm the efficacy and safety of this extract, it can be explored as an adjunct to traditional antibacterial treatments, with further possibilities related to application in personal care products, food preservation, or wound care. At the same time, it is illustrated that roselle extract could be a strong compound as an antibacterial agent against *S. epidermidis*, but to optimise its effect and form the specific active compounds and dosage and method of application, further studies are necessary.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# QUALITATIVE PHYTOCHEMICAL SCREENING AND ANTI-BACTERIAL ACTIVITIES OF ETHANOLIC MULBERRY LEAF EXTRACT (*MORUS* SPP.) AGAINST ISOLATED GRAM-POSITIVE BACTERIA

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**Abstract:** Mulberry (*Morus* spp.) leaves have long been recognized in traditional medicine and are now studied extensively for their antibacterial, antioxidant, anti-inflammatory, and antidiabetic properties. Their abundance in tropical and subtropical regions, including Malaysia, makes them an accessible and sustainable resource for health applications. The accelerating threat of antimicrobial resistance has highlighted the importance of plant-derived compounds as potential alternatives or complements to conventional antibiotics. However, systematic evaluation of mulberry leaf extracts against environmental bacterial isolates remains limited. This study aimed to identify the phytochemical constituents of ethanolic mulberry leaf extract and evaluate its antibacterial activity against Gram-positive bacteria isolated from different sources. Dried mulberry leaves were extracted in 70% ethanol, and the resulting crude extract was subjected to Fourier Transform Infrared (FTIR) spectroscopy and qualitative phytochemical assays. The antibacterial potential was assessed using the disc diffusion method against Gram-positive isolates collected from the toilet and laboratory door handles. Extract concentrations of 20, 15, and 10 mg/mL were tested alongside penicillin (positive control), ethanol (solvent control), and distilled water (negative control). FTIR and phytochemical tests confirmed the presence of bioactive compounds, including tannins, flavonoids, alkaloids, and terpenoids, which are known contributors to antimicrobial activity. Gram staining of isolates revealed purple cocci in clusters, consistent with Gram-positive *Staphylococcus* spp. Antibacterial assays demonstrated measurable inhibition zones across all extract concentrations, with the highest inhibition observed at 20 mg/mL (up to 5.49 mm). While inhibition zones were smaller than those of penicillin (14.30–23.95 mm), they exceeded those of solvent and negative controls, confirming bioactivity attributable to phytochemicals. In conclusion, ethanolic mulberry leaf extract exhibited modest but consistent antibacterial activity against Gram-positive environmental isolates, reinforcing its potential as a cost-effective, plant-based antibacterial source.

**Keywords:** *Mulberry leaf extract, Phytochemical, Antibacterial activity, Gram-positive bacteria, FTIR spectroscopy*

## INTRODUCTION

Mulberry (*Morus* spp.) has been cultivated for generations and is deeply embedded in traditional medical practices across Asia, particularly in China, Korea, India, and Southeast Asia (Chen et al., 2022; Ercisli & Orhan, 2007). Historically, mulberry leaves have been used to treat fever, cough, inflammation, and metabolic diseases (Chen et al., 2024; Morales Ramos et al., 2021). Contemporary pharmacological research confirms that mulberry leaves are rich in bioactive secondary metabolites, including flavonoids, alkaloids, tannins, terpenoids, and phenolic acids, which underpin a broad spectrum of biological activities such as antioxidant, anti-inflammatory, antimicrobial, and antidiabetic effects (Chen et al., 2024; Jelled et al., 2017; Lee & Hwang, 2017).

The global rise of antimicrobial resistance (AMR) poses a significant threat to human health, as multidrug-resistant bacteria increasingly undermine the effectiveness of standard antibiotics (World Health Organization, 2023). This challenge has driven interest in discovering new antimicrobials from natural, sustainable sources. In this context, the mulberry plant is rich in valuable phytochemicals that present a compelling alternative because it is widely available, cost-effective, and often multifunctional in its bioactivity (Batiha et al., 2023; Manzoor & Qayoom, 2024). Its accessibility makes it an attractive candidate for developing plant-based antibacterial agents.

Previous studies have established the antibacterial properties of mulberry extracts against clinically significant pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Latifah et al., 2024; Suriyaprom et al., 2021; Wahab et al., 2020). These effects are primarily attributed to polyphenolic compounds and flavonoids, which compromise bacterial membranes, inhibit nucleic acid synthesis, and impair enzyme activity (Daglia, 2012; Zahra et al., 2024). Nevertheless, most investigations have focused on standard laboratory strains, with comparatively fewer studies examining environmental bacterial isolates from high-touch surfaces that are potential reservoirs for pathogen transmission in community and hospital settings.

In light of the pressing demand for effective, low-cost antimicrobials and the phytochemical potential of mulberry leaves, this work evaluates the functional groups and phytochemicals in ethanolic mulberry leaf extract using FTIR spectroscopy and qualitative assays. Furthermore, its antibacterial efficacy was assessed against Gram-positive bacteria obtained from environmental sources, thereby evaluating its potential as a sustainable, plant-derived antibacterial agent.

## **MATERIALS AND METHODS**

### **Plant extraction.**

Dried mulberry leaf powder (25 g) was macerated in 70% ethanol (250 mL; 1:10 w/v) for 24 hours, stirred 2 h, filtered, and concentrated (heated to reflux; solvent removed at 175 atm). A stock (20 mg/mL in ethanol) was prepared with serial dilutions to 15 and 10 mg/mL.]

### **ATR-FTIR spectroscopy**

Fourier-transform infrared (FTIR) analysis was performed using an Attenuated Total Reflectance-FTIR (ATR-FTIR) spectrometer (Spectrum Two™, PerkinElmer). The spectrometer was equilibrated, and a background spectrum was recorded to remove ambient interference. Samples were then scanned over the 4000–400  $\text{cm}^{-1}$  range at a resolution of 4  $\text{cm}^{-1}$ , and the resulting spectra were interpreted to assign the functional groups present in the extract.

### **Phytochemical tests**

**Alkaloid's test:** A clean test tube was filled with 2 mL of 20 mg/mL mulberry extract, 0.2 mL of diluted hydrochloric acid (HCl), and 1 mL of Mayer's reagent. After gently stirring the mixture, an orange-brown precipitate formed, which indicated that the mulberry leaf extract contained alkaloids.

**Flavonoid test:** Two to three drops of sodium hydroxide were added to 2 mL of the 20 mg/ml mulberry extract in a clean test tube. A deep yellow colour appeared, but it gradually disappeared and became colourless when a few drops of dilute HCl were added, indicating that flavonoids were present.

**Terpenoid test:** In a tube test, 0.5 mL of the 20 mg/mL mulberry leaf extract solution was mixed with 2 mL of chloroform. After gently mixing the solution, 3 mL of concentrated sulphuric acid ( $\text{H}_2\text{SO}_4$ ) was added. The mixture was examined for the development of a reddish-brown interface between the sulphuric acid ( $\text{H}_2\text{SO}_4$ ) and chloroform ( $\text{CH}_2\text{Cl}$ ) layers after the two liquids separated into separate layers. This reddish-brown interface acted as confirmation that the extract contained terpenoids.

**Tannins' test:** A total of 2 mL of 5% iron (III) chloride ( $\text{FeCl}_3$ ) solution was added to a clean test tube that contained 5 mL of the 20 mg/mL mulberry extract solution. A greenish-black precipitate formed, indicating the presence of tannins.

### **Bacterial isolation from the environment**

Environmental swabs were collected from laboratory and toilet door handles, cultured on nutrient agar, and sub-cultured three times by streaking to acquire a pure colony. Gram staining was conducted and observed under light microscopy to confirm the presence of Gram-positive or Gram-negative bacteria for antibacterial activity.

### **Antibacterial testing**

Disc diffusion on nutrient agar inoculated with broth cultures (20  $\mu\text{L}$  spread per sector) compared 20, 15, and 10 mg/mL extract versus 70% ethanol (solvent control), penicillin (positive control), and distilled water (negative control). Paper discs (~6 mm) were soaked with test solutions; plates were incubated at 37°C for 18 h. Inhibition zone diameters were measured thrice with a Vernier calliper and averaged. The disc diffusion plates were repeated 2-3 times.

## **FINDINGS**

FTIR analysis of the ethanolic mulberry leaf extract revealed characteristic peaks corresponding to major phytochemical groups (Figure 1). These peaks are consistent with the presence of hydroxyl, aromatic, and nitrogen-containing functional groups, supporting the phytochemical richness of the extract. The qualitative phytochemical tests confirmed these findings (Table 1). Collectively, these results demonstrate that the mulberry leaf extract is rich in bioactive secondary metabolites, including tannins, alkaloids, flavonoids, and terpenoids, which are widely associated with antimicrobial, antioxidant, and anti-inflammatory activities. Flavonoids and phenolic compounds are frequently implicated in disrupting bacterial cell membranes and inhibiting the functions of enzymes and nucleic acids. The results presented here are consistent with the broader literature. In a recent

study, Kim et al. (2022) demonstrated that *Morus alba* leaf extract suppressed the growth of *Streptococcus mutans* and *Fusobacterium nucleatum*, likely via similar phenolic/flavonoid mechanisms (Kim & Kang, 2022).

Environmental swabs from both toilet and laboratory door handles yielded Gram-positive cocci upon staining (Figure 2). The cells appeared violet to purple, confirming their ability to retain crystal violet dye due to thick peptidoglycan cell walls. The cocci were predominantly arranged in clusters, a morphology typically associated with *Staphylococcus spp.* These findings validate the suitability of the isolates as test organisms for evaluating the antibacterial efficacy of the extract, consistent with previous findings (Latifah et al., 2024; Suriyaprom et al., 2021; Wahab et al., 2020).

The antibacterial effects of ethanolic mulberry leaf extract at concentrations of 20, 15, and 10 mg/mL were evaluated against the isolates (Table 2). All concentrations produced measurable inhibition zones, with diameters ranging from 1.97 to 5.49 mm. The 20 mg/mL concentration consistently yielded the largest inhibition zones, particularly against the laboratory door's knob isolate AL (5.49 mm), indicating a dose-dependent relationship. The 15 mg/mL and 10 mg/mL concentrations still exhibited inhibitory effects, albeit smaller in diameter, indicating a reduction in potency with dilution.

When compared to the solvent control (70% ethanol), the extract exhibited greater inhibition across most isolates, confirming that the activity was attributable to phytochemicals rather than residual ethanol. The negative control (distilled water) showed negligible inhibition ( $\leq 2.31$  mm), further supporting this conclusion. However, penicillin, the positive control, produced significantly larger inhibition zones (14.30–23.95 mm), highlighting the comparatively weaker antibacterial potency of the extract against environmental Gram-positive bacteria.

The modest inhibition observed suggests that white mulberry leaf extract does not rival the potency of standard antibiotics; its activity is biologically relevant and may be enhanced through higher concentrations, purification, or synergistic combination with antibiotics. Previous reports have similarly attributed the antibacterial action of mulberry extracts to phenolic compounds and flavonoids, which disrupt microbial membranes and interfere with metabolic pathways (Suriyaprom et al., 2021; Lee & Hwang, 2017).

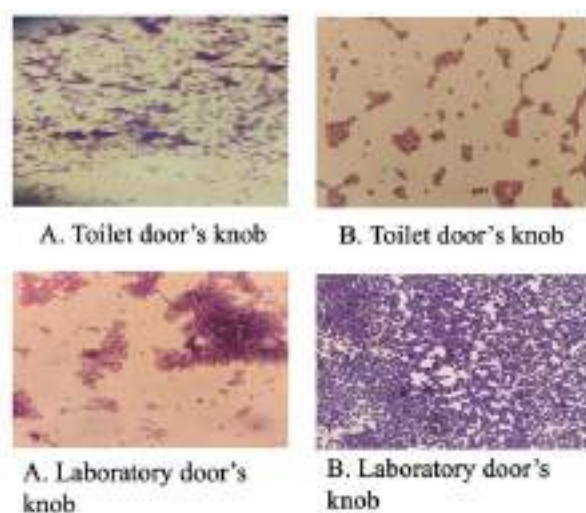
Our results show modest inhibition zones (maximum ~5.49 mm) for the highest extract concentration (20 mg/mL), whereas the standard antibiotic (penicillin) demonstrated significantly larger zones (~15–25 mm). This is in line with many plant extract studies, which show that extracts often require much higher concentrations to approach antibiotic efficacy. For example, Suriyaprom et al. (2021) reported that white mulberry extract showed activity against key foodborne bacteria. However, their MICs are generally low (~0.32–0.64 mg/mL for some species) compared to others (Suriyaprom et al., 2021). In our research, higher concentrations are required, suggesting that the crude extract has lower potency or contains fewer active compounds. Additional research should involve chromatographic fractionation, determine the minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC), and assess any potential synergistic effects with traditional antibiotics.



**Figure 1** FTIR peaks from ethanolic mulberry leaf extract

**Table 1** Overview of FTIR peaks and corresponding phytochemical tests with colorimetric outcomes from ethanolic mulberry leaves extract

| Test / Assay   | FTIR band(s) (cm <sup>-1</sup> )                   | Key features / significance   | Colour before test        | Colour change after test   |
|--|--|---|---------------------------|----------------------------|
| <b>Tannins test</b>                                  | 3339.67 (O–H), 1599.31 (aromatic C=C)              | Astringent; antioxidant; potential antimicrobial  | Light brown or colourless | Brownish or yellowish      |
| <b>Alkaloids (Mayer's)</b>                           | 1599.31 (N–H bending), 1258.54 (C–N stretching)    | Nitrogen-containing metabolites; antimicrobial, anti-inflammatory, neuroprotective          | Colourless                | Orange-brown precipitate   |
| <b>Alkaline reagent test (phenolics/ flavonoids)</b> | 3339.67 (O–H / phenolic)                           | Indicates phenolic compounds (tannins/flavonoids) with antioxidant & antimicrobial activity | Deep yellow               | Gradually turns colourless |
| <b>Terpenoids</b>                                    | 2933.40 (C–H stretching), 1258.54 (C–O stretching) | Bioactive; anti-inflammatory, anticancer, antimicrobial; present in essential oils          | Pale/ colourless          | Reddish brown              |



**Figure 2** Gram-stained bacterial isolates collected from door handles and visualized under a light microscope at 100× magnification. (A) Bacterial smear from a toilet door's knob showing Gram-positive cocci in clusters. (B) Another view of the toilet door's knob, isolated with densely aggregated Gram-positive cocci. (C) Laboratory door's knob isolate revealing Gram-positive cocci arranged in irregular clusters. (D) Additional smear from the laboratory door's knob displaying numerous Gram-positive cocci in dense aggregates. The purple/violet colouration indicates retention of crystal violet, confirming Gram-positive cell wall structure.

**Table 2** Antimicrobial activity of different concentrations of ethanolic extract of mulberry leaves on isolated bacteria from different sources

| Solutions testing on disc diffusion | Inhibition zone (mm)    |                         |                             |                             |
|-------------------------------------|-------------------------|-------------------------|-----------------------------|-----------------------------|
|                                     | Toilet Door's Knob (AT) | Toilet Door's Knob (BT) | Laboratory Door's Knob (AL) | Laboratory Door's Knob (BL) |
| Extract 20 mg/mL                    | 2.76                    | 3.45                    | 5.49                        | 1.97                        |
| Extract 15 mg/mL                    | 3.95                    | 3.54                    | 4.44                        | 4.49                        |
| Extract 10 mg/mL                    | 3.48                    | 2.94                    | 4.45                        | 3.09                        |
| Ethanol 70%                         | 2.33                    | 2.57                    | 5.01                        | 3.20                        |
| Penicillin                          | 23.95                   | 20.58                   | 21.82                       | 14.30                       |
| Distilled water                     | 1.56                    | 1.96                    | 2.31                        | 1.95                        |

## CONCLUSION

This study demonstrated that ethanolic mulberry (*Morus* spp.) leaf extract contains a diverse array of phytochemicals, including tannins, flavonoids, alkaloids, and terpenoids, as confirmed by FTIR analysis and qualitative assays. These bioactive compounds are widely reported to contribute to antimicrobial, antioxidant, and anti-inflammatory effects. The most excellent antibacterial activity was observed against Gram-positive cocci at a concentration of 20 mg/mL, indicating a dose-dependent effect. Although the inhibition zones were smaller than those produced by penicillin/streptomycin, they exceeded those of the solvent and negative controls, verifying that the activity was attributable to phytochemicals rather than residual ethanol. Overall, the findings highlight mulberry leaves as a readily available and cost-effective natural resource with modest antibacterial potential.

## COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

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# ANTI-DIABETIC POTENTIAL OF *Stenochlaena palustris* AND *Diplazium esculentum*

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**Abstract:** The increasing global prevalence of diabetes mellitus highlights the urgent need for alternative therapeutic strategies that are affordable, effective and associated with minimal side effects. This study aimed to comparatively assess the anti-diabetic potential of *Stenochlaena palustris* and *Diplazium esculentum* ethanolic extracts through phytochemical screening and in-vitro bio-assay. The crude ethanolic extracts of both ferns were subjected to standard qualitative phytochemical analysis and anti-diabetic activity was evaluated using  $\alpha$ -glucosidase inhibition assay. Both *S. palustris* and *D. esculentum* extracts demonstrated the presence of bioactive phytochemicals, with flavonoids, phenolics, saponins and tannins compounds being dominant. Comparative enzyme inhibition assay revealed that *S. palustris* and *D. esculentum* demonstrate moderate and comparable  $\alpha$ -glucosidase inhibitory activity. It can be concluded that *S. palustris* and *D. esculentum* possess promising anti-diabetic potential. Further in-vivo and animal trials are warranted to validate their efficacy and safety as phytopharmaceutical candidates.

**Keywords:** *Phytochemicals, Ferns, Hyperglycemic, Diabetes mellitus, Metabolic disorder*

## INTRODUCTION

Diabetes mellitus is a complex, chronic metabolic disorder characterized by persistent hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. It has emerged as one of the most pressing global health challenges of the 21st century, affecting an estimated 783 million adults worldwide by 2045 (Hossain et al., 2024). The condition is broadly classified into type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM). T1DM is an auto immune mediated destruction of pancreatic  $\beta$ -cells leading to absolute insulin deficiency (Aamodt and Powers, 2025). T2DM, which accounts for more than 90% of cases and is primarily associated with insulin resistance, progressive  $\beta$ -cell dysfunction and lifestyle factors (Gieroba et al., 2025). Gestational diabetes mellitus and other specific forms, including monogenic and secondary diabetes, further contribute to its heterogeneity. Consequently, diabetes mellitus is a leading cause of cardiovascular disease, chronic kidney disease, blindness and lower limb amputations, imposing substantial health and economic burdens globally (Alijanzadeh et al., 2024).

Despite advances in therapeutic strategies, including insulin formulations, oral hypoglycaemic agents and novel classes such as GLP-1 receptor agonists and SGLT2 inhibitors, the prevention and management of diabetes remain major challenges (Kang et al., 2025). Current research emphasizes not only pharmacological interventions but also lifestyle modifications and public health strategies aimed at reducing risk factors and delaying disease progression. Understanding the epidemiology, underlying mechanisms, diagnostic and therapeutic approaches to address this growing global health concern (Caturano et al., 2024). Beside significant advancements in synthetic anti-diabetic therapies, challenges persist in terms of cost, accessibility, adverse side effects and patient compliance. These limitations have fuelled a growing interest in alternative and complementary strategies, particularly plant-derived therapeutics.

In recent years, extensive pharmacological and clinical studies have provided scientific validation of numerous plants with anti-diabetic potential, such as *Momordica charantia* (bitter melon), *Trigonella foenumgraecum* (fenugreek), *Zingiber officinale* (ginger) and *Curcuma longa* (turmeric) (Sayem et al., 2025). The increasing body of evidence supports their role not only on glycaemic control but also in ameliorating diabetic complications, including dyslipidaemia, nephropathy and cardiovascular dysfunction. Given the escalating global diabetes mellitus burden and the limitations of existing therapeutic options, plant-based anti-diabetic treatments represent a promising avenue for drug discovery, integrative medicine and sustainable healthcare strategies (Ahmad et al., 2025). Therefore, this study was carried out to explore the potential of two ferns, native to Malaysia, namely *Stenochlaena palustris* ('paku miding', 'paku naga' or 'pakis merah' in Malay and climbing swamp fern in English) and *Diplazium esculentum* ('pucuk paku', 'paku tanjung' or 'paku besar' in Malay and vegetable fern in English).

## METHODOLOGY

### Plant materials and ethanolic extraction

Aerial parts of *Stenochlaena palustris* and *Diplazium esculentum* were collected from their natural environments in Behrang, Perak and authenticated by a plant taxonomist at Center of Biodiversity and Conservation, Universiti Pendidikan Sultan Idris. The plant materials were thoroughly washed, shade dried at room temperature and pulverized into coarse powder using an electrical blender. Approximately 100 g of the powdered plant material was macerated in 1 L of 80% ethanol (1:10 w/v) for 72 h at room temperature with occasional agitation. The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted twice under the same conditions to ensure maximum yield. All filtrates were pooled and concentrated under reduced pressure using a rotary evaporator at 40 °C to remove the solvent. The concentrated crude extract was then dried to constant weight in the oven at 40 °C and stored in airtight container at 4 °C until further analysis.

### Phytochemical screening

Qualitative phytochemical screening of the extracts was performed using standard procedures as described by Salunke et al. (2025). The presence of major secondary metabolites including phenols, flavonoids, saponins, tannins, alkaloids, terpenoids, triterpenes glycosides and steroids were assessed through characteristic colorimetric changes and precipitation reactions. All procedures were carried out in triplicate to confirm the reproducibility of results, and observations were recorded based on the intensity of the colour change or precipitate formation.

### $\alpha$ -glucosidase inhibition assay

The  $\alpha$ -glucosidase inhibitory activity of the extracts was evaluated using a method of Rosa et al. (2025) with some modifications. Briefly, 50  $\mu$ l of sample solution at various concentrations was pre-incubated with 100  $\mu$ l of 5 mM p-nitrophenyl- $\alpha$ -D-glucopyranoside (pNPG) prepared in phosphate buffer (pH 6.8) at 37 °C for 5 min. The reaction was initiated by adding 50  $\mu$ l of  $\alpha$ -glucosidase enzyme solution (0.5 U/ml in phosphate buffer, pH 6.8). After incubation at 37 °C for 20 min, the reaction was terminated by adding 100  $\mu$ l of 0.1 M sodium carbonate. The absorbance of the resulting p-nitrophenol was measured at 405 nm using a microplate reader. Acarbose was used as the positive control, while buffer served as the blank. The percentage of inhibition was calculated as:

$$\text{Inhibition (\%)} = [(A_c - A_s) / A_c] \times 100$$

Where  $A_c$  is the absorbance of the control and  $A_s$  is the absorbance of the sample.

### Statistical analysis

All data are subjected to one-way ANOVA and values of  $p < 0.05$  were considered statistically significant.

## FINDINGS

The qualitative phytochemical screening of the aerial parts of *Stenochlaena palustris* and *Diplazium esculentum* ethanolic extracts revealed the presence of several bioactive compounds (Table 1). Phenols, flavonoids, saponins, tannins and triterpenes glycosides compounds were detected in varying intensities, indicating the rich secondary metabolite content of *S. palustris* and *D. esculentum* ethanolic extracts. Terpenoids and steroids were not detected in both extracts. Alkaloids were detected in *S. palustris* ethanolic extract but not detected in *D. esculentum* ethanolic extract. The abundance of phenols and flavonoids constituents suggests strong antioxidant potential, whereas the presence of saponins, tannins, triterpenes glycosides and alkaloids may contribute to diverse pharmacological activities (Belew and Gebre, 2025). These findings highlight the therapeutic relevance of the extract and provide a foundation for further quantitative and bioactivity-guided studies.

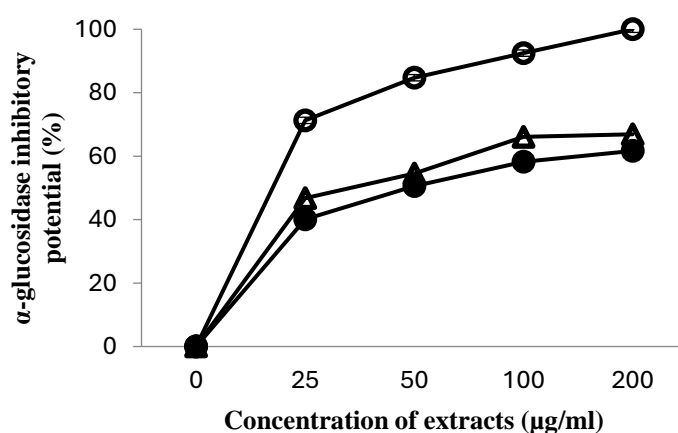
**Table 1** Phytochemical constituents of *S. palustris* and *D. esculentum* aerial parts ethanolic extracts.

| Phytochemical          | <i>Stenochlaena palustris</i> | <i>Diplazium esculentum</i> |
|------------------------|-------------------------------|-----------------------------|
| Phenols                | ++                            | ++                          |
| Flavonoids             | ++                            | +                           |
| Saponins               | +                             | ++                          |
| Tannins                | ++                            | ++                          |
| Alkaloids              | ++                            | n/d                         |
| Terpenoids             | n/d                           | n/d                         |
| Triterpenes glycosides | +                             | ++                          |
| Steroids               | n/d                           | n/d                         |

Indicator: (++) high intensity detected, (+) low intensity detected, (n/d) not-detected

The ethanolic extracts of *Stenochlaena palustris* and *Diplazium esculentum* were evaluated for their in-vitro anti-diabetic activity using the  $\alpha$ -glucosidase inhibition assay. Both extracts exhibited dose-dependent inhibitory effects

(Figure 1), suggesting the presence of bioactive compounds capable of modulating carbohydrate metabolism or insulin secretion. However, statistical analysis revealed no significant differences ( $p > 0.05$ ) in the inhibitory potential between the two species, indicating that both ferns possess comparable anti-diabetic activity. Results and statistical analysis also revealed that anti-diabetic activity of *S. palustris* and *D. esculentum* significantly lower ( $p < 0.05$ ) compared to Acarbose, a synthetic anti-diabetic drug commonly used in the management of type 2 diabetes mellitus and belongs to the class of  $\alpha$ -glucosidase inhibitors. Qualitative phytochemical screening supported *S. palustris* and *D. esculentum* anti-diabetic potential, as both extracts demonstrated the presence of phenols, flavonoids, saponins and tannins which are highly reported for their anti-diabetic properties (Velmurugan et al., 2025). Flavonoids and phenolics in particular, are known to exert inhibitory effects on carbohydrate-hydrolysing enzymes and enhance glucose uptake (Hatanaka et al., 2024). Meanwhile, saponins and tannins contribute synergistically to glycaemic regulation (Babar et al., 2025). The similarity in phytochemical constituents between *S. palustris* and *D. esculentum* ethanolic extracts may explain their equivalent inhibitory responses in-vitro. Overall, these findings support the traditional consumption of *S. palustris* and *D. esculentum* as functional vegetables with health benefits, particularly in glycaemic control. Future studies involving in-vivo models and compound isolation are warranted to further validate their efficacy and elucidate the specific metabolites responsible for the observed effects.



**Figure 1**  $\alpha$ -glucosidase inhibition activity of Acarbose (○) and ethanolic extract of *Stenochlaena palustris* (Δ) and *Diplazium esculentum* (●) aerial parts.

## CONCLUSIONS

The comparative evaluation of the ethanolic extracts of *Stenochlaena palustris* and *Diplazium esculentum* demonstrated that both ferns possess comparable anti-diabetic potential, as evidenced by their similar  $\alpha$ -glucosidase inhibitory activity. Phytochemical screening revealed overlapping profiles, with both extracts containing phenolics, flavonoids, saponins and tannins, which are likely contributors to the observed bioactivity. Although minor differences in phytochemical intensity and constituents were noted, these variations did not translate into significant differences in anti-diabetic efficacy. It can be concluded that both ferns are promising sources of natural  $\alpha$ -glucosidase inhibitors. Further in-vivo studies and bioactive compound isolation are recommended to validate and expand upon these results.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# PHYTOCHEMICAL SCREENING AND ANTI-OBESITY ACTIVITY OF ZINGIBERACEAE RHIZOME ETHANOLIC EXTRACT

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**Abstract:** The prevalence of obesity as a global health concern has prompted increasing interest in plant-based therapeutics with bioactive potential. This study aimed to comparatively evaluate the phytochemical profiles and anti-obesity activities of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes ethanolic extracts. Qualitative screening technique was used to determine their phytochemical constituents and pancreatic lipase inhibition assay was used to determine their anti-obesity capacities. All three extracts revealed abundant phytoconstituents, with variation in intensity, suggesting diverse bioactive potentials. Anti-obesity results indicated that *Z. officinale* ethanolic extract exhibited moderate pancreatic lipase inhibitory effect, while *C. longa* and *A. galanga* showed low but consistent activity across the assays (dose dependent response). These findings suggest that rhizomes from the Zingiberaceae family possess anti-obesity potential at differing levels. Further investigations on isolation of active compounds and animal trials are recommended.

**Keywords:** *Zingiber officinale*, *Curcuma longa*, *Alpinia galanga*, Pancreatic lipase, Spices, Secondary metabolites

## INTRODUCTION

Globally, the prevalence of obesity is rising alarmly in both industrialized and developing nations (Ahmed and Mohammed, 2025). An imbalance between energy intake and expenditure leads to obesity. A range of obesity medications that affect energy expenditure or appetite have been utilized in the past; however, they have all been shown to have major adverse effects. For example, the US Food and Drug Administration ordered the removal of anti-obesity synthetic drug, lorcaserin, from the market due to a safety clinical trial that revealed a higher incidence of cancer (Zheng, 2025). Currently, a number of plants have been examined for their anti-obesity properties, but because of their significant cytotoxicity, very few of them are used in practice.

The Zingiberaceae family, commonly known as the ginger family, is one of the largest families of monocotyledonous flowering plants. There are approximately 52 genera and 1300 species in the Zingiberaceae family, which includes *Zingiber officinale* Roscoe, *Curcuma longa* Linn and *Alpinia galanga* Linn (Poojita and Nandha, 2025). They were extensively dispersed throughout the continents of America, Africa and Asia. The rhizome of Zingiberaceae is a rich source of different classes of compounds that belong to a wide variety of chemical metabolites, such as polyphenols, alkaloids and terpenes (Wable et al., 2025). Members of this family have been widely used in traditional medicines, culinary practices and sources of natural bioactive compounds. They also exhibit significant pharmacological activities such as antioxidant, anti-inflammatory, anti-microbial and metabolic regulatory activities (Paudel et al., 2025).

Given their ethnopharmacological importance and promising bioactivities, several studies have been conducted to investigate the anti-obesity potential of Zingiberaceae species. However, comparative studies evaluating the phytochemical composition and relative anti-obesity efficacy of this family remain limited. Therefore, in the present study, the comparative analysis of phytochemical constituents and the anti-obesity of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes ethanolic extracts were *in-vitro* evaluated using pancreatic lipase inhibition assay.

## METHODOLOGY

### Plant materials and ethanolic extraction

*Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes were obtained from local garden. Taxonomic authentication was carried out by the Herbarium of Universiti Pendidikan Sultan Idris, Malaysia. The plant materials were coarse powdered and macerated with 80% ethanol. The extracts were collected every 72 hours for

three times and were concentrated by using rotary evaporator (Yulianti et al., 2025). Concentrated crude extracts were kept in the refrigerator at 4 °C in sealed containers for further use.

### Qualitative phytochemical screening

The qualitative screening for phenols, flavonoids, saponins, tannins, alkaloids, terpenoids, triterpenes glycosides and steroids were performed by using standard protocols as previously described (Latha et al., 2025).

### Pancreatic lipase inhibition assay

The assay was carried out as previously described (Mohanty and Yadav, 2025). Two hundred µl of orlistat as a synthetic drug or plant extracts (0, 25, 50 and 100 µg/ml) were incubated with 100 µl of pancreatic lipase solution and 700 µl of Tris-HCL solution (pH 7.4) for 15 min at 25 °C. Then, 100 µl of p-nitrophenylbutyrate in dimethyl formamide was added and the mixture was further incubated for 30 min at 37 °C. Lipase inhibition activity was measured at 405 nm using a UV-visible spectrophotometer. The percentage of lipase inhibition was calculated according to the following formula:

$$\text{Inhibition (\%)} = [1 - (B - b) / (A - a)] \times 100$$

Where A is the activity of the enzyme without the inhibitor, a is the negative control without the inhibitor, B is the activity of the enzyme with the inhibitor, and b is the negative control with the inhibitor.

### Statistical analysis

All data are subjected to one-way ANOVA and values of p<0.05 were considered statistically significant.

## FINDINGS

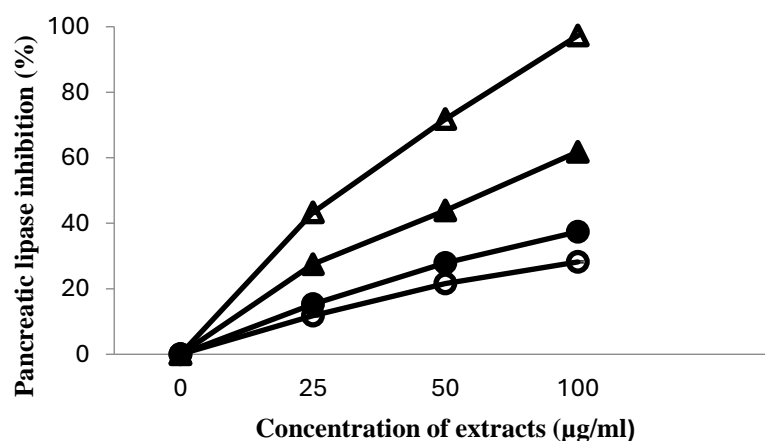
The phytochemical screening of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes ethanolic extracts are summarized in Table 1. Results revealed the presence of key secondary metabolites including phenols and flavonoids, although with varying intensities. The ethanolic extracts of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes ethanolic extracts also showed the presence of saponins, tannins, alkaloids and terpenoids, but showed negative result for the presence of triterpenes glycosides and steroids. Previous authors reported that phenols, flavonoids and terpenoids influence lipid metabolism, inhibit fat absorption and enhance energy expenditure (Subas et al., 2025).

**Table 1** Phytochemical constituents of *Z. officinale*, *C. longa* and *A. galanga* rhizomes ethanolic extracts.

| Phytochemical          | <i>Zingiber officinale</i> | <i>Curcuma longa</i> | <i>Alpinia galanga</i> |
|------------------------|----------------------------|----------------------|------------------------|
| Phenols                | ++                         | +                    | +                      |
| Flavonoids             | ++                         | +                    | +                      |
| Saponins               | +                          | +                    | +                      |
| Tannins                | +                          | +                    | +                      |
| Alkaloids              | +                          | +                    | +                      |
| Terpenoids             | ++                         | +                    | +                      |
| Triterpenes glycosides | n/d                        | n/d                  | n/d                    |
| Steroids               | n/d                        | n/d                  | n/d                    |

Indicator: (++) high intensity detected, (+) low intensity detected, (n/d) not-detected

The inhibitory activity of Orlistat and ethanolic extract of *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes against pancreatic lipase are shown in Figure 1. Ethanolic extract of *Z. officinale* rhizome exhibited moderate pancreatic lipase inhibitory activity, and significantly lower (p<0.05) compared to Orlistat, a synthetic drug that served as a positive control. Among the Zingiberaceae, *Z. officinale* exhibited the strongest pancreatic lipase inhibitory activity (p<0.05), followed by *C. longa* and *A. galanga* rhizomes ethanolic extracts. The results also showed that all three extracts inhibited the activity of pancreatic lipase in a dose-dependent response at differencing levels.



**Figure 1** Pancreatic lipase inhibition activity of Orlistat ( $\Delta$ ) and ethanolic extract of *Zingiber officinale* ( $\blacktriangle$ ), *Curcuma longa* ( $\bullet$ ) and *Alpinia galanga* ( $\circ$ ) rhizomes.

Previous findings attributing gingerols and shogaols in *Z. officinale* to anti-obesity activity via lipid metabolism regulation (Preciado-Ortiz et al., 2025). Gingerols are the major phenolic compounds in fresh ginger, while shogaols are formed when gingerols undergo dehydration, often during processing or storage (Samota et al., 2024). Even though in the current study, *C. longa* demonstrated low pancreatic lipase inhibitory activity, previous author reported that *C. longa* exhibited strong antioxidant activity which may contribute indirectly to obesity management by reducing oxidative stress associated with adipogenesis (Marina et al., 2025). *A. galanga* also showed relatively lower inhibitory activity but maintained consistent effect across assays, suggesting synergistic contributions of its phytochemicals. Overall, the observed variations in pancreatic lipase inhibitory activity among the three species are closely related to differences in phytochemical composition and intensity, particularly the abundance of flavonoids, phenolics and terpenoids.

## CONCLUSIONS

The comparative analysis *Zingiber officinale*, *Curcuma longa* and *Alpinia galanga* rhizomes ethanolic extracts revealed that all three species are rich in bioactive phytochemicals with potential anti-obesity effects. Among them, *Z. officinale* demonstrated the strongest pancreatic lipase inhibitory activity, followed by *C. longa* and *A. galanga*. These findings highlight the therapeutic promise of Zingiberaceae rhizomes as natural anti-obesity agents and provide scientific support for their traditional use and suggest their potential role in the development of safe plant-based interventions for obesity management. Further studies focusing on the isolation of active compounds, mechanistic pathways and animal trials are warranted to validate and optimize their applications.

## COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

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# INFLUENCE OF PITFALL TRAP FUNNEL ORIENTATION ON INSECT ABUNDANCE IN TRAP CROP

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**Abstract:** Pitfall trap is a widely used method in ecological research for passive sampling of ground-dwelling insects. The efficiency of a pitfall trap can be influenced by many physical factors, including the trap design. The present study was conducted to determine the influence of funnel orientation of the pitfall trap for the insect collection at a banana crop site, a type of trap crop, at Universiti Teknologi MARA, Perak Branch, Tapah Campus. The sampling was performed using different designs of funnel orientations, i.e., upward funnel, downward funnel and multiple funnels, with the standard design without a funnel served as the control group. The abundance of collected insects was analysed using Microsoft Excel 2007. Based on the results, the pitfall trap effectively captured a diverse assemblage of insect orders, with Orthoptera (40%), Hymenoptera (21%) and Diptera (13%) being the most common orders. Overall, 21 families representing 11 orders were recorded, with Gryllidae (20.51%), Acrididae (10.99%) and Formicidae (10.79%) identified as the most common families. The influence of different funnel orientations indicated that the downward funnel captured a higher abundance of insects than the other funnel orientations. However, the result also indicated that the control group of the pitfall trap (no funnel) captured more insects than the treatment groups. These findings suggest that optimisation in trap design can further improve data accuracy in ecological studies. This study provides practical suggestions for the improvement of biodiversity monitoring techniques in tropical ecosystems.

**Keywords:** *passive sampling, biodiversity, ground-dwelling arthropods, trap crop*

## INTRODUCTION

Pitfall traps are the best-known passive sampling technique and most often used inventory method for ground-dwelling insects. They have been widely used to explore the habitat quality (Ramalingam & Dharma Rajan, 2020) and for measuring nature conservation values (Jelaska, 2022). Pitfall traps usually consist of a beaker that is buried so that the lip of the beaker is level with the ground surface. This trap operates on the principle that once insects fall into the trap, they are unable to escape by climbing the trap walls and are subsequently preserved in the alcohol placed at the base. In previous study by Woodcock (2005), pitfall traps efficiently collect certain insect orders, i.e., rove beetles (Coleoptera: Staphylinidae), wandering spiders (e.g. Aranae: Lycosidae and Clubionidae), and ants (Hymenoptera: Formicidae). A major challenge in ecological and entomological studies is the lack of standardization across pitfall trap designs. Reviews have documented extensive variation in trap diameter, depth, preservative fluid, cover presence and spacing (Brown et al., 2016). This heterogeneity reduces comparability among studies and may introduce sampling biases (Conway & Hohbein, 2018). Additionally, one of the other parameters that has received relatively little attention is the funnel orientation of the pitfall trap. Differences in orientation may alter the probability of capture or escape, thereby biasing abundance estimates.

Without an empirical understanding of how funnel orientation affects capture efficiency, sampling data may be inconsistent, unreliable, or non-comparable across experiments or locations. Gaining insights into how trap design affects sampling efficiency is therefore essential for enhancing the accuracy and reliability of ecological monitoring in trap cropping systems. It is hypothesised that different funnel orientations will affect the number of insects captured in pitfall traps, with certain orientations enhancing trap efficiency compared to the standard design. Therefore, the objectives of the present study were to: i) determine the insect abundance within the trap crop, and ii) compare the influence of different funnel orientations of the pitfall trap on insect abundance.

## METHODOLOGY

### Sampling site

The field study was carried out at a banana crop site located within Universiti Teknologi MARA, Tapah Campus, Perak (Figure 1). The banana crop functioned as a trap crop and served as the focal site for insect sampling. The sampling was conducted every week from November to December 2024 (n=8 sampling sessions).



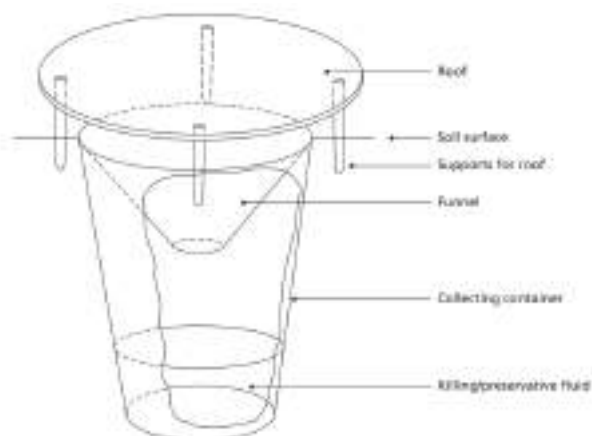
**Figure 1** Sampling site at Universiti Teknologi MARA, Tapah Campus, Malaysia

### **Pitfall trap design**

The pitfall traps were developed based on the proposed design by Woodcock (2005), as in Figure 2, with modifications to incorporate different funnel orientations. Four treatments were tested, i.e., no funnel (control), upward funnel, downward funnel, and multiple funnels. Each trap was assembled using readily available materials, including water bottles, bamboo sticks, duct tape, cable ties, hot glue and scissors. The water bottles served as the main trap body and were cut and shaped according to the required funnel orientation. The components were secured using duct tape, cable ties, and hot glue to ensure stability and durability in the field. Pitfall traps were deployed in a linear sequence consisting of 5 traps positioned at intervals of 1.5 to 2 m as proposed by Egorov et al. (2024).

### **Data analysis**

The collected insects from each sampling session were counted and morphologically identified using stereomicroscope to the order and family level with reference to available taxonomic keys and relevant literature (Heckman, 2006; Gibb & Oseto, 2019). Data analysis was conducted using Microsoft Excel 2007 to address the study objectives.



**Figure 2** Pitfall trap with funnel (Woodcock, 2005)

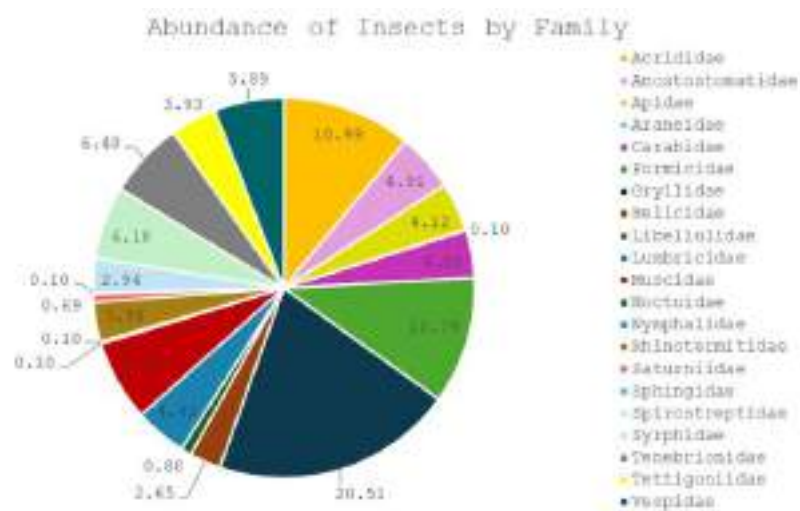
## **FINDINGS**

A total of 1,019 insects were captured from the banana crop site during the eight sessions over the two-month sampling period. The finding shows that the most common orders were Orthoptera (40%), Hymenoptera (21%) and Diptera (13%). In total, 21 families representing 11 orders were recorded, with Gryllidae (20.51%), Acrididae (10.99%) and Formicidae (10.79%) being the most common families (Figure 3). The diverse of insect orders collected from the banana crop can be attributed to several ecological and environmental factors. Banana plants provide an ideal habitat with dense foliage and complex structural features, offering shelter from predators and create suitable microhabitats for oviposition, both of which are crucial for their survival and reproduction (Padmanaban & Mani, 2022). Moreover, the availability of food resources within the area of the banana crop site plays a crucial role in supporting insect populations, particularly Gryllidae (crickets) and other Orthoptera. The

banana plants themselves serve as a primary food source, offering tender leaves and decaying plant matter that are rich in nutrients. These insects are known to feed on various parts of the banana plant, including the leaves, flowers, and fruits, which provide essential sustenance for their growth and reproduction (Sarkar et al., 2018).

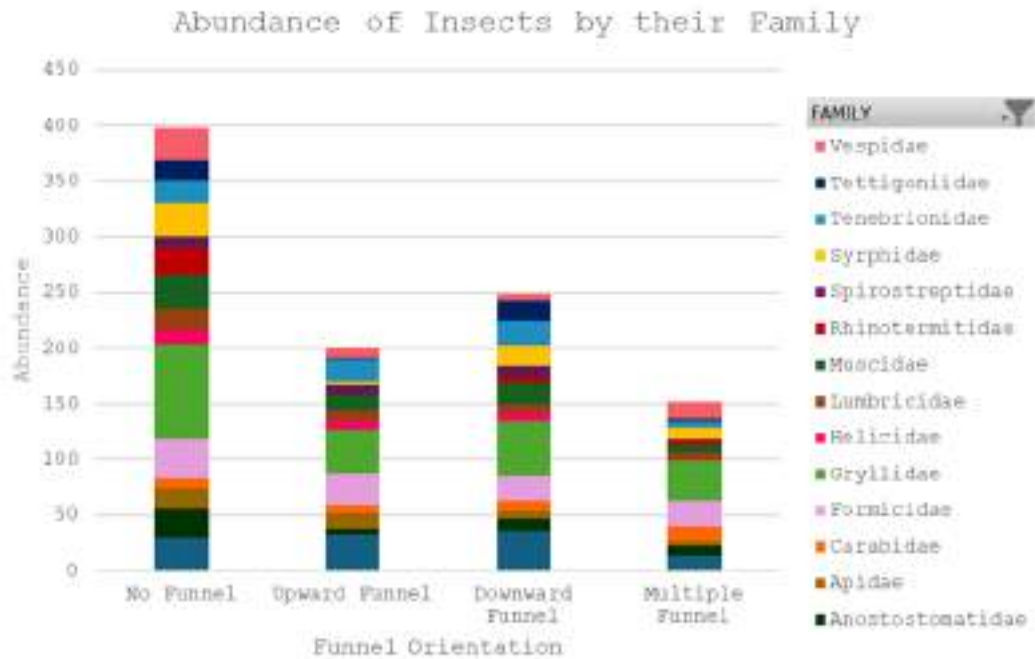
Apart from the abundance of insects, the abiotic metadata were also being recorded throughout the sampling sessions. Across the eight sessions, the temperature ranged from 28 °C to 34 °C, with an average of 31.38 °C. Light intensity values ranged from 6 to 10 lux, while humidity levels varied between 81.90% and 94.54%, with an average of 89.28%. Additionally, atmospheric pressure ranged from 1009.7 to 1012.2 hPa, while the average rainfall over the sampling period was 247.9 mm, with a maximum of 399.8 mm recorded. In previous study, the microclimatic conditions found in banana plantations are marked by a high humidity and stable temperatures, which are particularly conducive for the growth and development of crickets (Kuo & Fisher, 2022). With an average temperature of 31.38 °C and humidity levels around 89.28% in the present study, these conditions help maintain moisture levels that are essential for the physiological needs of these insects.

Furthermore, variations were observed in the abundance of insects representing different ecological roles within the banana trap crop. The collected insects were categorised into six ecological roles, i.e., beneficial insects, biocontrol agents, decomposers, pests, pollinators and predators. Most of the insects were classified as decomposers (445 individuals), followed by pests (239 individuals) and predators (221 individuals). In terms of ecological guilds, herbivores, i.e., crickets, grasshoppers and honeybees were the most abundant, followed by detritivores, including millipedes, earthworms, flies and termites and omnivores such as ants and spiders. The remaining insects were categorised as carnivores, consisting mainly of predatory beetles (e.g., Carabidae) and wasps.



**Figure 3** Abundance of insects by family

Moreover, the abundance of insects captured in pitfall traps varied with funnel orientation (Figure 4). As a result, 405 insects were captured from the control trap (no funnel), 256 insects from the downward funnel, 204 insects from the upward funnel and 154 insects from multiple funnels. The absence of a funnel in the control trap provided an open and unrestricted trap entrance, making it easier for a wide range of insects to fall in (Brown & Matthews, 2016). Next, by evaluating the different funnel orientations, the pitfall trap with a downward funnel successfully collected the highest abundance of insects, indicating superior trapping efficiency. This orientation likely capture success by guiding insects into the trap while preventing the entry of larger insects and reducing escape rates (Ahmed et. al, 2023). The upward funnel followed, capturing 204 insects, which represents a moderate abundance. The reduced capture rate in this orientation may be attributed to the narrow upper opening, which likely limited entry for certain insect taxa. In contrast, the multiple-funnel trap recorded the lowest abundance, with only 154 insects collected. The lower capture rate in this design could be due to the structural complexity of multiple funnels, which may have restricted access to the trap entrance and impeded insect entry (Hohbein & Conway, 2018).



**Figure 4** Abundance of insects by different funnel orientation

## CONCLUSIONS

This study revealed that funnel orientation influences the efficiency of pitfall traps in capturing ground-dwelling insects within a banana trap crop system. Different insect orders used the banana crop as their preferred habitat due to the complexity of the landscapes and the suitable climatic conditions, which promote the survival of the insects. Among the treatments, the downward funnel successfully captured relatively more insects than other funnel orientations; however, the conventional pitfall trap without a funnel (control group) recorded the highest overall abundance. This suggests that additional funnel structures may alter insect movement and accessibility to the trap entrance, thereby affecting capture efficiency. Orthoptera, Hymenoptera, and Diptera were the dominant orders, indicating that the traps effectively sampled a broad range of insect taxa typical of tropical agroecosystems. Future studies should extend sampling across multiple crop types, assess additional trap design parameters such as colour, size, and preservative type, and incorporate behavioural observations to better understand insect responses to trap geometry. Integrating pitfall traps with complementary sampling methods could also enhance data accuracy and provide a more comprehensive understanding of insect communities in tropical agricultural systems, especially in the trap crop site.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# ANTIBACTERIAL ACTIVITY OF HAEMOLYMPH EXTRACTED FROM *Coccinella septempunctata* AGAINST *Escherichia coli*: A PRELIMINARY STUDY

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**Abstract:** This study investigated the antibacterial activity of haemolymph extracted from *Coccinella septempunctata* against bacteria *Escherichia coli*. The rising issue of antibiotic resistance has encouraged the search for alternative natural antibacterial sources, including from insect haemolymph, which contains antimicrobial peptides (AMPs). In this study, haemolymph was extracted and tested using the agar well diffusion method on nutrient agar inoculated with *E. coli*. Four (4) treatment groups were prepared by mixing haemolymph (H) with buffer A (BA) (pH 6), B (BB) (pH 6.5), C (BC) (pH 5) and distilled water (DW) respectively. The results showed that haemolymph combined with BB resulted in the largest inhibition zone (5 mm), followed by BA and BC (4 mm), while combination with DW showed the smallest inhibition zone (2 mm). These findings suggest that haemolymph from *C. septempunctata* potentially exhibits antibacterial properties against *E. coli*, with buffer composition affecting its efficacy. Thus, the results highlight the potential of haemolymph derived from insects as a natural antimicrobial agent for future study.

**Keywords:** *Coccinella septempunctata*, *Escherichia coli*, Haemolymph, Antibacterial, Inhibition zone

## INTRODUCTION

Antibiotic resistance has emerged as one of the global health threats, compromising the efficacy of conventional antibiotics and increasing the urgency to discover novel antimicrobial agents (World Health Organization, 2020). As bacteria such as *E. coli* evolve to resist commonly used drugs, the search for alternative antibacterial sources has shifted toward natural and sustainable bioresources. Among these, insects have shown growing scientific interest due to their innate immune systems, which produce potent AMPs within their haemolymph, a circulatory fluid like vertebrate blood (Morejon & Michel, 2023).

*C. septempunctata*, commonly known as the seven-spotted ladybird, is a beneficial predatory beetle of the family Coccinellidae, widely recognized for its ecological role in pest control. Its haemolymph, which is abundance in immune-related compounds and defensive enzymes, has been suggested to possess antimicrobial properties that can inhibit bacterial growth. Studies on insect-derived AMPs from species such as *Hermetia illucens* and *Philosamia ricini* have demonstrated inhibitory effects against various pathogens (Singma et al., 2025). However, scarce evidence exists regarding the antibacterial potential of *C. septempunctata* haemolymph.

Exploring the bioactivity of *C. septempunctata* haemolymph is therefore relevant, not only to expand scientific understanding of insect immune defenses but also to identify natural bioactive molecules that could be developed into sustainable antimicrobial agents. This preliminary study investigates the antibacterial activity of haemolymph extracted from *C. septempunctata* against *E. coli*, to provide fundamental insights into the species' potential as a source of natural antibacterial compounds.

## METHODOLOGY

Adult specimens of *C. septempunctata* were manually collected from a garden within the Universiti Teknologi MARA (UiTM) Tapah Campus, Perak. The insects were maintained under controlled laboratory conditions until the haemolymph extraction process.

Buffers (BA, BB, and BC) were prepared under sterile conditions with varying pH and compositions as below.

- BA (pH 6.0): NaCl, glucose, trisodium citrate, citric acid
- BB (pH 6.5): NaCl, glucose, trisodium citrate, citric acid, Na<sub>2</sub>EDTA
- BC (pH 5.0): NaOH, NaCl, EDTA, citric acid

Haemolymph was extracted from live adult ladybirds by making a small incision using a sterile needle. The exuding fluid was immediately mixed with one of the prepared buffers to prevent clotting, collected with a micropipette, and stored in sterile microcentrifuge tubes on ice. The mixtures were gently vortexed to ensure homogenization.

A fresh culture of *E. coli* was prepared by inoculating a single colony into nutrient broth and incubating at 37°C for 18–24 hours. The optical density (OD) was adjusted to 0.143 to standardize bacterial concentration. Nutrient agar plates were prepared, autoclaved, and solidified under aseptic conditions.

The antibacterial assay was performed using the agar well diffusion method. Wells of 6 mm diameter were created in agar plates seeded with *E. coli* using a sterile cork borer. Each well received 20 µL of the haemolymph-buffer mixture. A standard antibiotic served as the positive control (PC), while sterile distilled water served as the negative control (NC). Plates were incubated at 37 °C for 24 hours, after which zones of inhibition were measured in millimeters to evaluate antibacterial activity. Each treatment was conducted in triplicates, and mean inhibition zone diameters were calculated.

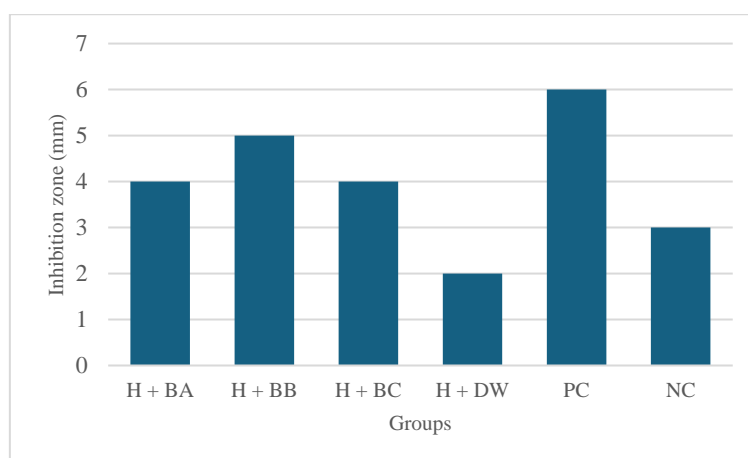
## FINDINGS

The findings from this preliminary study demonstrated that the haemolymph extracted from *C. septempunctata* exhibited antibacterial activity against *E. coli*, thus supporting the hypothesis that insect haemolymph contains bioactive compounds capable of inhibiting bacterial growth. The variation in inhibition zone diameters among the treatments indicates that buffer composition significantly influenced haemolymph stability and antibacterial efficacy. The combination of haemolymph with buffer B (BB) (pH 6.5, containing Na<sub>2</sub>EDTA) resulted the largest inhibition zone diameter (5 mm), suggesting that a slightly alkaline environment and the presence of chelating agents such as EDTA may enhance the antimicrobial activity by maintaining protein integrity and destabilizing bacterial cell membranes (Lee et al., 2021).

In contrast, haemolymph mixed with distilled water produced the smallest inhibition zone diameter (2 mm), possibly due to protein denaturation. Buffers A (BA) and C (BC) showed moderate inhibition zone diameter (4 mm). This suggests the importance of balanced ionic composition and pH to maintain the functionality of antimicrobial peptides (AMPs). These findings align with previous studies reporting that the bioactivity of insect haemolymph is influenced by environmental factors and buffer systems (Caccia et al., 2019; Morejon & Michel, 2023).

The antibacterial effect observed can be attributed to the presence of AMPs and immune-related proteins within the haemolymph. AMPs such as cecropins, defensins, and attacins are small, cationic peptides known to disrupt bacterial cell membranes and interfere with essential metabolic pathways (Bulet & Stöcklin, 2005; Ruiu, 2015; Wu et al., 2018). Insects produce these peptides as part of their innate immune defense, often regulated through signaling cascades such as the Toll and Imd pathways (Hoffmann, 2022; Hanson & Lemaitre, 2020). The measurable inhibition of *E. coli* growth therefore confirms that *C. septempunctata* haemolymph contributes to innate immune defense and may possess novel AMPs with therapeutic potential (Sahoo et al., 2023).

Furthermore, the positive control (antibiotic) exhibited a 6 mm inhibition zone, validating the sensitivity of *E. coli* under the assay conditions. Although the antibacterial activity of haemolymph was lower than that of the synthetic antibiotic, its efficacy remains notable given that it was unpurified and tested in crude form. With proper purification and concentration optimization, stronger antibacterial effects could likely be achieved, as demonstrated in similar studies involving purified peptides from *Hermetia illucens* and *Harmonia axyridis* and other insect species (Röhrich C.R et al., 2012; Park et al., 2015).



**Figure 1** Antibacterial activity of different combination of haemolymph from *C. septempunctata* (H) and buffer A (BA), B (BB), C (BC) and distilled water (DW).

## CONCLUSIONS

This preliminary study demonstrated that the haemolymph extracted from *C. septempunctata* possesses antibacterial activity against *E. coli*. Buffer B enhanced its activity the most, suggesting the importance of buffer composition in maintaining the stability of antibacterial compounds. The results highlight the potential of insect haemolymph as a natural source of antimicrobial agents. Its bioactive compounds could be developed into sustainable alternatives against conventional antibiotics, reducing dependency on synthetic antimicrobials and mitigating the spread of antibiotic resistance. Future studies should focus on isolating and characterizing the bioactive compounds responsible for these effects and expanding tests to wider range of bacterial species.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# EVALUATION OF RED ROSE EXTRACT AS A NATURAL DYE: A FOUR-METHOD APPROACH TO COLOR VISION ANALYSIS

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**Abstract:** Due to their environmentally benign qualities, natural dyes are growing ever more popular as long-term substitutes for synthetic dyes. This study investigates the extraction of natural dye from rose petals and its application to cotton fabric, aiming to evaluate its colour vision. Rose petals were chosen for their abundant availability and potential as a sustainable dye source. As concerns about the negative effects of synthetic colorants on the environment and human health grow, natural dyes offer low toxicity, biodegradability, and renewable sourcing. Four extraction techniques (acetic acid, sodium hydroxide, ethanol and distilled water) were applied to fresh rose petals, and the extracted dyes were tested on cotton fabric. The color properties of the dye extracted from rose petals varied significantly depending on the extraction medium. Acidic extraction yielded the most vibrant and stable purple hue. In contrast, alkaline extraction resulted in a dull orange-brown tone that faded over time. Aqueous extraction produced the lightest color, a pale pink, while alcohol-based extraction generated a medium brown shade. These findings suggest that the extraction medium plays a crucial role in determining both the intensity and stability of the color obtained from rose petals. These results are in keeping with earlier studies that demonstrated anthocyanin pigments are more stable when extracted in an acidic environment but deteriorate when extracted in an alkaline environment.

**Keywords:** *Natural dye, Extraction technique, Dried rose petals, Colour vision*

## INTRODUCTION

The use of synthetic dyes in the textile industry has caused major health and environmental issues because of their toxicity, lack of biodegradability, and detrimental ecological effects. On the other hand, natural dyes provide environmentally benign substitutes, although they frequently have poor fastness qualities, uneven repeatability, and low colour yields (Pizzicato et al., 2023). The extraction of natural dyes is highly influenced by the solvent medium, which affects both the yield and the shade of the dye. Common solvents used in rose petal include acetic acid (acidic medium), sodium hydroxide (alkaline medium), ethanol (organic solvent), and distilled water (aqueous medium). Acidic media like acetic acid help stabilize anthocyanins and enhance red to purple hues, while alkaline extraction often leads to pigment degradation, producing dull brownish shades (Salman et al., 2023; Sadaf et al., 2024). Ethanol is effective in extracting both polar and moderately non-polar compounds, yielding deeper brownish or reddish tones (Patil, 2016). Due to their vivid colours and bioactive qualities, rose petals have become a popular and environmentally responsible natural dye. The pigments in question, which are primarily anthocyanins and include molecules of the malvidin, pelargonidin, cyanidin, peonidin, and delphinidin types, are soluble in water and react with pH and binding interactions to change colour. (Iwashina, 2015). In this study, the extraction is performed across four different solvent systems (acid, base, alcohol, aqueous), followed by cotton dyeing under controlled settings. The colour vibrant from the extraction is observed. In practical applications, rose derived anthocyanins have shown considerable potential in textile dyeing. A 2021 study reported successful extraction of anthocyanin dyes from *Rosa indica* petals, achieving varied shades on wool fabrics using both chemical and bio-mordants to enhance colour intensity and fastness. Notably, these dyed fabrics also displayed antioxidant, anti-inflammatory, and antibacterial properties, underscoring roses' multifunctional value in textile applications (Adeel et al., 2021).

## METHODOLOGY

### Preparing and scouring of cotton clothes

Cotton fabric (2.5 x 2.5 inches) was placed in a 200 ml beaker and boiled with 100 ml of 10% (w/v) sodium hydroxide solution for 15 minutes to remove starch and other impurities from the cloth. Then, the cotton cloths were thoroughly washed with cold distilled water to remove any remaining impurities. (Grover & Patni, 2011)

### Aqueous extraction method

10 g of fresh red rose petals was boiled at 100 °C for 30 minutes in 100 ml distilled water. The decolorized petals were taken out from extraction solvent. (Patil et al., 2016).

### Alkaline extraction method

10 g of fresh red rose was added in 100 ml of 1% (w/v) sodium hydroxide solution and boiled at 100 °C for 30 minutes. The decolorized petals were removed from the extraction solvent (Patil et al., 2016).

### Acidic extraction method

10 g of fresh red rose petals was added in 100 ml of 1% (v/v) acetic acid solution. The mixture was boiled at 100 °C for 30 minutes. The decolorized petals are removed from the extraction solvent (Patil et al., 2016).

### Alcohol extraction method

10 g of fresh red rose petals was added in 100 ml of 50% (v/v) alcohol (ethanol) solution and boiled at 100 °C for 30 minutes. The decolorized petals were removed from the extraction solvent (Patil et al., 2016).

### Dyeing method

A (2.5 x 2.5 inch) cotton fabric was immersed in the beaker with the extracts. After 30 minutes, the fabric was removed and left to dry for one day to observe the intensity of the colours.

## FINDINGS

The different colour shades were obtained from various extracts of red rose petal. The extracts show variation in colour, and which mainly depends upon the extraction solvents. Based on the observation, cotton fabric immersed in acetic acid extraction produces a vivid purple colour in 30 minutes showing it has high and strong pigmentation. In comparison, cotton fabric in sodium hydroxide extraction shows dull orange-brown shade. Figure 1 shows the appearance of colour shade in four different extractions.

The extraction from ethanol on cotton fabric shows muted light brown with low brightness and moderate intensity while aqueous extraction forms a pale pinkish colour dye. All results are compared to each other, and it was found that acid has a strong effect on cotton fabric. In this research it is found that red rose colour shade changes properties when in acidic it gives strong shade on cotton fabric as compared to the aqueous, alcohol and alkaline extraction. Rose petals are rich in anthocyanins, a group of water-soluble flavonoid pigments responsible for red, pink, purple, and blue hues in plants (Wang et al., 2022). Anthocyanins are highly pH-sensitive, and their structural transformations under varying pH directly influence the color of extracts (Gonzalez-Barrio et al., 2009). Anthocyanins exist in several structural forms depending on pH like at acidic pH ( $\text{pH} < 3$ ), anthocyanins predominantly exist as flavylium cations, which absorb light strongly and appear vivid red or pink (Brouillard & Dangles, 1994). In a study by Sadaf et al. (2024), rose petals were extracted using acidic, alkaline, and aqueous methods and applied to cotton fabric.



**Figure 1** Dyeing process of cotton fabric

The acidic extraction, which likely involved acetic acid, produced the most vibrant and persistent purple colour on the fabric. This indicates that the acidic environment enhances the extraction and stability of anthocyanins, resulting in deeper and more durable hues. While at neutral pH, the pigments undergo hydration and form colourless carbinol pseudobases or pale chalcone structures, this leads to a lighter, more muted tone, often pale pink rather than vivid red or purple (Brouillard & Dangles, 1994; Salman et al., 2023). According to a study by Al-Alwani et al. (2023), wool textiles dyed with rose petal extract at an acidic pH (pH 2-3) exhibited excellent

binding and noticeably higher colour strength than those dyed at a neutral or basic pH. The study on 2023 by Salman et al, four distinct solvent systems aqueous (neutral), acidic (pH = 2), alkaline (pH ≈ 11), and basified methanol have been used to study the extraction of rose petal pigments for use in wool dyeing. Out of all of them, the acidic extraction procedure, which was carried out at 40°C for 35 minutes while adding 2 g/100 mL of salt, showed the greatest tint (K/S) values (Kubelka-Munk theory), indicating higher dye uptake and stronger colour strength. This implies that, in contrast to neutral, alkaline, or methanol-based extraction techniques, acidic conditions promote superior stability of anthocyanin pigments, resulting in more vivid pigmentation (Salman et al., 2023).

This research also indicate that cotton is a good fabric for dyeing as it has high absorbency power, so it absorbs dye. Cotton is a natural fabric so applying natural dye on it is ecofriendly too. Cotton due to high absorbency power resist shade change and staining properties in colour fastness to wash and colour fastness to light test (Sadaf et al., 2024).

## CONCLUSIONS

This study successfully retrieved colour from rose petals with four different solutions: aqueous, alkaline, acidic, and alcohol. The results showed that the acidic extraction procedure generated the most brilliant and stable colour, whilst the alkaline, alcohol, and aqueous extractions produced increasingly weaker hues. Red rose flower extracts serve as an economical and environmentally friendly source of natural dyes, with wide applicability in sectors such as textiles, cosmeceuticals, leather tanning, and food coloring.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# THE ELECTROCHEMICAL POTENTIAL OF A NATURAL GREEN BATTERY FROM FRUITS

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**Abstract:** A battery is an electric cell, a device that produces electricity from a chemical reaction used for many purposes. Commercial batteries often contain toxic materials that can contaminate water sources and cause harm to the environment due to improper disposal. As the pollution alarms, this study investigates the potential of fruits as a green alternative to commercial batteries. The objectives of this study are to compare the pH and voltages of variations of fruits, to determine the difference in voltage between fresh fruit, non-fresh fruit and blended fruit and to study the potential of fruits as an alternative to the battery for a decoration product created. The methodology begins with the pH identification of four variations of fruits; lemon, orange, green apple and pineapple. Then, the voltage of the fruits was measured using copper and zinc as electrode. A product decoration in flower form is created with the fruit juice as a green battery. The resulted pH of lemon was measured is 3 and pH 4 is for orange, green apple and pineapple. Fresh fruit juice produced higher voltages than non-fresh and blended samples, with lemon, orange, green apple and pineapple yielding 2.08 V, 1.48 V, 1.76V and 1.22 V, accordingly. Meanwhile, the light-emitting diode (LED) lighted up the brightest when using lemon fruit compared to other fruits. These findings demonstrate that fresh lemon juice is the most effective fruit-based electrolyte for biobattery applications, offering higher voltage output and better performance for powering small decorative devices compared to orange, green apple and pineapple.

**Keywords:** Battery, Green battery, Fruit, electricity, Voltage, Energy

## INTRODUCTION

Batteries are essential energy sources which contains two plates made of dissimilar metals as electrodes that are immersed in a conducting solution called as electrolytes. According to Hill et al. (2005), chemical reactions occur when an electron flows from cathode to anode through an electrolyte. This is called as an oxidation-reduction reaction or known as a redox reaction transform chemical energy into electrical energy (Chang et al., 2013). Commercial battery production increased by day and improper disposal leads to the polluted environment. The materials in alkaline commercial batteries are corrosive acids like cadmium, lead and mercury that will risk the ecosystem and contamination of soil and water sources. Hence, an eco-friendly and biodegradable power supply has become an important focus by encouraging the society to use green batteries derived from natural materials. Fruit based electrochemical cell will help to reduce the chemical waste contain in an alkaline battery that cannot be recycled. The effect of pollution and greenhouse will reduce and safe to the environments (Garcia et al., 2003).

The introduction of fruits is naturally acidic, being a source of battery that can generate electricity. In recent years, green batteries derived from natural resources and used food from fruits or vegetables. The natural organic acids in the fruit's juices provide electrolytes that facilitate ion transfer between electrodes thus triggering a chemical reaction (Sagar et al., 2010). Usually, the acidic fruits are used because of high acidity. Therefore, acid is the producer of electrons in producing electricity (Garcia et al., 2003). There are several factors that affect the conduction of electric in fruits which are pH and the condition of the fruits or vegetable. The pH affects the voltage obtained from the chemical reaction between the electrodes and the electrolytes. Meanwhile, the condition of the fruit was determined based on its physical state and its stage of decomposition (Klotz et al., 2003).

This study investigated the electrochemical potential of the different types of fruits as alternative energy sources. The difference in pH and voltage output under different conditions which are fresh, non-fresh and blended between types of fruits were measured. The potential application as a green battery in powering decorative product was studied. Fresh lemon produced the highest voltage of 2.08V and LED lighted up brightly in decorative product compared to the other fruits.

## METHODOLOGY

The methodology consisted of three main parts; pH and voltage determination, and application of fruits as a green battery. Four different fruits were focused involving lemon, orange, green apple and pineapple. First, the pH value of each fruit was determined using pH paper. Each fruit was cut and squeezed to extract the juice and a pH paper was dipped. The pH paper was observed and compared against the standard pH scale to obtain the pH value. Next, the voltage of each fruit was measured. Lemon was cut using a knife, where the slits matched for the plate to be inserted. A copper plate and a zinc plate were inserted halfway into each of the slits, opposite of each other. Both copper and zinc plate were connected together with a set of wire and alligator clips, where the other ends were connected to a multimeter (Sagar et al., 2010). The voltage reading was recorded and the same steps were repeated for other fruits. The steps were repeated for fruit juice aged for a day and blended juice. The final procedure involved the application of the fruit as a green battery for the decoration product created. The electrolytes from fruit need to power up the LED on a decoration. The decoration was initially constructed by cutting a piece of coloured paper into several pieces, with the shape of a flower petal. The copper wire and galvanised wire were then cut into several pieces, each about 5 cm in length. A piece of the copper wire was slipped alternately into the holes of a petal and galvanised wire at the opposite side. The copper wire on the petal was twisted with the galvanised wire on another petal and continued until five petals for a flower were comprised. The copper wire of the fifth petal was twisted to an LED instead and the same goes for the galvanised wire of the first petals. The petals of the flower were drenched with juice. In order to make the light of the LED observable, the decoration flower was placed inside a cardboard box wrapped with black coloured paper.

## FINDINGS

The experimental results were obtained from measuring the pH and voltage output of four different fruits under fresh, non-fresh, and blended conditions. As shown in Table 1, lemon exhibit the lowest pH value which was 3 and pH 4 for orange, green apple and pineapple. Lower pH value of lemon indicated that lemon has high concentration of acidity from organic acid. According to Karadeniz (2004) the highest acid content was found in lemon juices compared to other fruits, with 58.94 g/L corresponding to citric acid. Based on the sample fruit tested, lemon and orange were categorized as citrus fruits that had high amount of citric acid. However, pH orange is 4 due to less concentration of citric acid than lemon. Nevertheless, green apple and pineapple are not classified as citrus fruits and contain organic acid primarily malic acid more than citric acid. However, both fruits are still acidic.

**Table 1** The pH and voltage of variation condition of fruits

| Types of fruit | pH | Voltage, V  |                 |               |
|----------------|----|-------------|-----------------|---------------|
|                |    | Fresh juice | Non fresh juice | Blended juice |
| Lemon          | 3  | 2.08        | 1.96            | 1.43          |
| Orange         | 4  | 1.48        | 1.42            | 1.04          |
| Green apple    | 4  | 1.76        | 1.69            | 1.21          |
| Pineapple      | 4  | 1.22        | 1.12            | 0.86          |

*Reference: Mohd Noor, 2016*

For the voltage output, lemon produced the highest voltage compared to others. Higher acidity in lemon produced more voltage. Citric acid acts as electrolytes in the battery, increasing the strength of the electric current and thereby the voltage produced (Akbar et al., 2018). This shows a correlation that the lower pH of the fruit directly contributes to the higher voltage produced (Sultana et al., 2018). The more citric acid present in acidic fruit, the more hydrogen ions being produced to react with the electrons on both electrodes, generating higher current flow (Sangaranarayanan et al., 2011).

Table 1 also revealed that the fresh fruits consistently produced the highest voltage for each type of fruits compared to non-fresh fruit and blended fruit. Fresh lemon exhibited the highest voltage of 2.08V, followed by green apple, orange and pineapple with 1.48V, 1.76V and 1.22V accordingly. Citric acid in lemon enhances conductivity and resulted in greater voltage generation, which is supported by a previous study where acid is a good conductor of electrons. This created an electric current that can be used as bio battery (Sasongko et al., 2025). While the orange produced more voltage than the pineapple but less than green apple, the pH remained similar to that of the apple despite its higher citric acid content. This may be attributed by the variations in acid concentration, fruit size and surface area. By comparing fresh and non-fresh juice, a decrease in voltage was observed with ageing as shown in Table 1. Lemon fresh juice produced higher voltage 2.08V compared to 1.96V for non-fresh fruit. Similar trends were observed for orange, green apple and pineapple. The lowest voltage was pineapple in non-fresh juice condition. Research by Sasongko et al. (2025) emphasized that the freshness of the fruits depends on its storage or preservation time which is known as aging time. The fresh and aged fruits can

indicate their biochemical and electrochemical state. These values are influenced by factors such as the chemical composition, ripeness, water content, and the degradation of organic compounds during ageing. In addition, voltage values obtained from all the fruits juice electrolyte reduce with time. This is probably due to the oxidation process on the electrolyte. Lemon and orange juice electrolyte decreases with time, possibly due to oxidation (Osahenvenwen et al. 2020). The biochemical degradation process affects the decrease in voltage. In comparison with all conditions, blended juice produced the lowest voltage among the three conditions, with pineapple being the lowest at 0.86 V. The decreased voltage may be due to the disruption of the natural fruit structure during blending, which limits efficient electron transfer between the electrodes. Thus, fresh juices produced higher voltages than aged samples.

Additionally, the application of fruit juices in powering a decorative LED flower and the electrochemical performance as a green battery electrolyte was discussed. For the application, Table 2 summarizes the qualitative performance of lemon, orange, and pineapple juices showing the brightness level of the LED as an indicator of each fruit's effectiveness as a natural green battery.

**Table 2** The condition of LED on the application of natural green battery

| Types of Fruit | Condition of LED          |
|----------------|---------------------------|
| Lemon          | The LED light up brightly |
| Orange         | The LED light up          |
| Green apple    | The LED light up          |
| Pineapple      | The LED light up          |

*Reference: Mohd Noor, 2016*

Table 2 revealed the result of the different types of fruit that act as a natural green battery in flower decoration product that has been made. Among the four fruits tested, lemon produced the brightest light, indicating that its higher voltage output translated into more effective current flow through the LED circuit as shown in Figure 1. Meanwhile, orange, green apple and pineapple were able to light up the LED, but with visibly lower brightness, reflecting their lower voltage values.



**Figure 1** The condition of the LED when the petals is implemented with fresh lemon juice

Based on the finding, this confirms that the lemon is the most potential fruit electrolyte due to its higher acidity and citric acid concentration, which enhance conductivity and electron transfer between electrodes. The performance difference also highlights that voltage strength is directly correlated with LED brightness, making lemon juice the best candidate for the green battery applications among the fruits studied. Overall, lemon provides the highest voltage among all the electrolytes used and the most effective material for being as bio green battery.

## CONCLUSIONS

From the study, the pH of a fruits does affect the value of the voltage obtained. Comparing all the types of fruit, fruits with higher acidity produced higher voltage outputs, with lemon as pH 3 generating the greatest voltage among the fruits tested with 2.08V, followed by orange, green apple and pineapple. The acidity affects the higher voltage obtained. Fresh lemon juice was identified as the most potential electrolyte, producing a higher voltage compared to non-fresh and blended juices. Fresh juices produced higher voltages than aged sample because electron transfer was more efficient, whereas blending disrupted the internal structure and reduced conductivity. For the application as a green battery, a decorated flower utilizing fresh lemon juice produced a brighter LED illumination compared to orange juice, green apple and pineapple juice. Nevertheless, further research is needed to optimize this alternative battery system for practical use as a replacement for conventional alkaline batteries. Future work should explore different electrode materials, acid concentrations and methods to preserve juice

freshness over time. Additionally, improvements to the green battery prototype are necessary to enhance its efficiency and stability.

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# PRELIMINARY STUDY ON THE USE OF WASHED RICE WATER AS A NATURAL FERTILIZER FOR GERMINATION AND GROWTH OF WATER SPINACH

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**Abstract:** Washed rice water (WRW), the starchy liquid left after washing rice, is rich in essential nutrients such as nitrogen, phosphorus, potassium, and beneficial microbes. This study evaluates the effectiveness of WRW as a sustainable and eco-friendly alternative to chemical fertilizers in promoting seed germination and early plant development. Water spinach (*Ipomoea aquatica*) seeds were pre-soaked and planted in black soil, with three treatment groups; tap water (control), fresh rice water, and aged (three days) rice water. Each treatment was replicated, and plants were watered every two days over 30 days. Growth parameters including germination rate, plant height, and number of leaves were measured. The results show that aged rice water achieved a 90% germination rate, along with the greatest leaf production and plant height, compared to fresh rice water and the control. This suggests that aged rice water could serve as an affordable and sustainable substitute for synthetic fertilizers, making it particularly advantageous for small-scale farmers and home gardeners. The study contributes to sustainable agricultural practices by promoting WRW as a low-cost, environmentally friendly fertilizer for improving crop yields and plant health.

**Keywords:** *Washed rice water, Aged rice water, Water spinach, Seed germination, Plant growth*

## INTRODUCTION

Agriculture remains a fundamental pillar of global food security and economic development, providing sustenance, employment, and income for millions worldwide. In Malaysia, agriculture remains a key contributor to the national economy, accounting for approximately 8% of the country's gross domestic product (GDP) in 2022 (DOSM, 2023). While its relative share of GDP has declined over the decades due to industrialization and service-sector growth, agriculture continues to play a critical role in rural development, food production, and trade. Chemical or synthetic fertilizers have played a crucial role in boosting global food production. However, long-term reliance on mineral fertilizers often alters soil chemistry and biology in ways that undermine sustainability. Continuous nitrogen fertilization has been shown to acidify soils, lowering pH and causing nutrient imbalances that reduce productivity over time (Howe, 2024). Indirectly, the use of chemical fertilizer also driven health risks where groundwater contaminated with nitrates from excessive fertilization has been linked to methemoglobinemia and possible carcinogenic effects (Madjar et al., 2024). Natural fertilizers including farmyard manure, compost, crop residues, green manures, biofertilizers, and other organic amendments are increasingly recognized as sustainable alternatives or complements to chemical fertilizers. Das et al. (2022) showed that compost and manure applications increase soil carbon levels, improve aggregation, and stabilize pH which can promote better root growth and water retention, which are critical under drought conditions.

With growing emphasis on sustainable agriculture, both small-scale gardeners and farmers are adopting environmentally friendly practices to improve soil fertility and crop performance. One promising approach is the use of washed rice water (WRW), a household byproduct often discarded as waste but known to contain valuable plant nutrients. Rice is available in multiple forms, including rough rice, brown rice, parboiled rice, conventionally milled white rice, pre-cooked rice, quick-frozen rice, and products such as puffed, crisped, or expanded rice (Abbas et al., 2011). The mineral profile of rice is influenced by factors such as varietal differences, fertilization practices, cultivation conditions, processing and cooking methods, as well as the characteristics of the soil in which it is cultivated (Abbas et al., 2011; Roy et al., 2011). The objective of this study is to investigate the effects of WRW including fresh and aged rice water on seed germination rates and subsequent plant growth. WRW provides essential macronutrients such as nitrogen, phosphorus, and potassium, along with trace minerals that support plant growth and microbial activity. Although short-term studies highlight its potential as a low-cost biofertilizer, the long-term agronomic and ecological impacts of WRW application are still not fully understood (Nabayi et al., 2021). Additionally, this study is significant for fully utilizing resources, particularly discarded materials like rice water. By exploring its nutrient content and potential for plant growth, the research also aims to reduce waste and promote sustainability.

## METHODOLOGY

### Preparation of Seeds and Soil

Water spinach (*Ipomoea aquatica*) seeds and black soil, serving as the growing medium, were obtained from the local market. The seeds were randomly selected and pre-soaked in tap water for six hours before planting.

### Preparation of Treatment Solutions

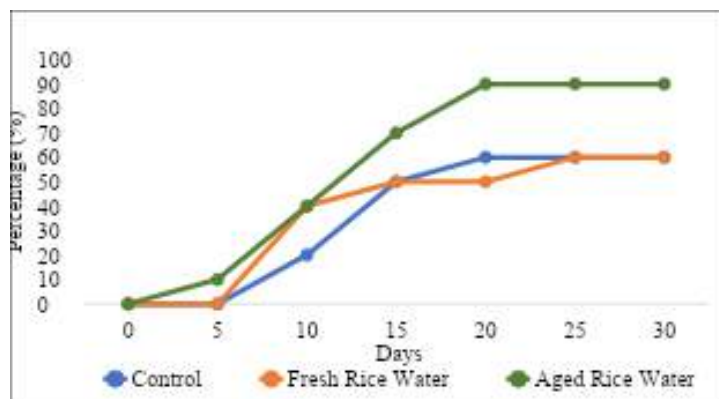
Two types of rice water were prepared as treatment solution which are fresh rice water and aged rice water. Fresh rice water was obtained by rinsing one cup of uncooked rice in two cups of water for 10 minutes, after which the water was immediately strained to extract the nutrients. Aged rice water was prepared using the same procedure and the strained water was stored in a 500 mL closed container at room temperature for three days to allow natural fermentation.

### Evaluation of the Treatment Solutions

Six pots were prepared, each containing 10 seeds planted in the same type of growing medium, and divided into three treatment groups, with two pots in each group. The control group will be watered with tap water, the fresh rice water group will receive freshly made rice water and the aged rice water group will be given rice water stored for three days. Each treatment was made into a duplicate. All pots were put under direct sunlight at the same time every day and watered with 100 mL of their respective solutions every two days from the time of planting. Watering was done in the morning to minimize evaporation loss and allow plants to utilize the moisture effectively throughout the day. Once seedlings emerged, the water amount was increased gradually to 200 mL every two days to support growth. The pots were observed daily for 30 days. An outdoor setting with steady sunlight and few fluctuations in the environment was used for this study. Apart from that, in the preparation of aged rice water, the 500 mL closed container containing the aged rice water will be kept at room temperature for three days before use. The effectiveness of treatment solutions was assessed every 5 days based on germination rate and plant growth by plant height and number of leaves.

## FINDINGS

The study evaluated the potential of rice water as a natural fertilizer through three treatments: control, fresh rice water, and aged rice water. Plant performance was assessed based on germination rates, plant height, and leaf number, recorded at five-day intervals over a 30-day period. Each treatment group was treated with its respective solution every two days. Germination rates refer to the proportion of seed that germinate under the tested condition (Ghaleb et al., 2022). Figure 1.0 illustrates the germination rates of water spinach seeds throughout the study.

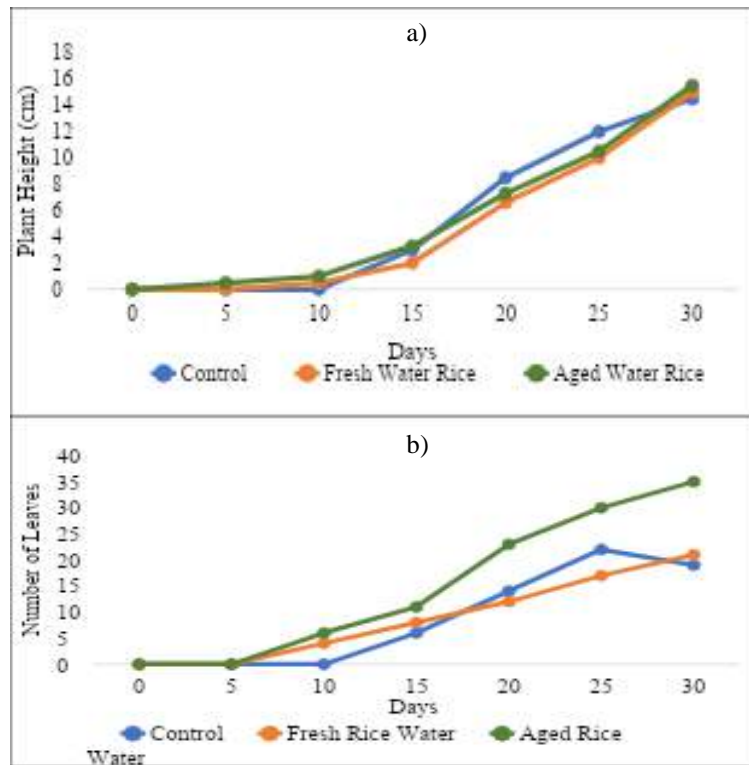


**Figure 1** Germination rates for three different treatments group

In general, the germination rates increase over time across for all treatments, with aged rice water showing highest germinated seed at 90% compared to control and fresh rice water at 60%. Aged rice water showed earliest seed germinated among the treatment groups at day 5. Rice water contains nutrients like carbohydrates, proteins, and minerals, along with small amounts of growth-promoting compounds which can support seed germination and early seedling growth (Sharma et al., 2020). Additionally, the presence of organic acids produced during fermentation of rice water could contribute to an improved soil environment by enhancing nutrient solubility and availability (Nabayi et al., 2023).

Plant growth is commonly evaluated using a combination of morphological parameters, such as plant height, number of leaves, stem diameter, and root length, as well as biomass measurements including fresh and dry weight of shoots and roots (Taiz et al., 2015). In this study the plant growth were assessed based on plant height and

number of leaves. Figures 2a) and 2b) present the plant height and number of leaves of water spinach recorded over a 30-day observation period respectively.



**Figure 2** Plant growth a) plant height b) number of leaves

Figure 2a illustrates the plant height (cm) observed over a 30-day period, where plants treated with aged rice water achieved the greatest height of 15.5 cm compared to the other two treatments. Notably, only the aged rice water treatment showed measurable growth by day 5, while no growth was recorded in the other treatments at that stage. On the other hand, Figure 2b shows the number of leaves produced, where rice water treatments resulted in leaf formation after 10 days. Plants treated with fresh rice water developed four leaves, while those treated with aged rice water produced six leaves. In contrast, the control group did not exhibit any leaf development in 10 days. At 30 days after treatment, water spinach treated with aged rice water exhibited the highest leaf count (35 leaves), compared to those treated with fresh rice water (21 leaves) and the control group (19 leaves).

The results of this study demonstrate that aged rice water markedly enhanced seed germination and plant growth compared to both fresh rice water and the control. Plants receiving aged rice water exhibited the highest germination rate along with superior growth performance. Fermentation converts complex organic compounds into simpler, more readily available forms. For rice water, this process can release amino acids, micronutrients, and beneficial microorganisms that promote seed germination. According to Nabayi et al. (2021), fermented washed rice water enhances populations of plant growth-promoting bacteria, which support both germination and overall plant vigor. These microbes may secrete enzymes and hormones, such as gibberellins, that play a key role in breaking seed dormancy and stimulating faster germination.

## CONCLUSIONS

This research examined the possible uses and advantages of WRW on germination rate and plant growth. Both fresh and aged rice water improved germination rate and plant growth in water spinach compared to the control, with aged rice water showing the most pronounced effect. Given its nutrient-rich composition, WRW represents a sustainable resource with multiple applications, including as an agricultural fertilizer, a natural cleaning agent, and a component in cosmetic formulations. Its reuse also contributes to reducing food waste and promoting environmental sustainability. Nonetheless, further research is needed to optimize its applications through detailed analyses and well-structured experiments, including investigations of microbial activity, pH, and temperature effects. Overall, the findings advance current knowledge of WRW's potential and encourage further investigation for practical use in diverse sectors.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# ASSESSMENT OF HEAVY METAL CONTENTS IN ANCHOVIES USING ATOMIC ABSORPTION SPECTROSCOPY

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**Abstract:** This study was designed to assess the concentration of iron (Fe) and cadmium (Cd) in anchovies by using Atomic Absorption Spectroscopy (AAS) across four different shops (TF Mart, Segi Fresh, 100 Mart and Pasar Mini Tapah) around Tapah, Perak. This research also aims to assess the potential health risks of these metals by calculating the Hazard Index (HI) values. Sample preparation involved drying the anchovies in an oven at 60°C and then grinding them into a fine powder, which was sieved for uniformity. The digestion process was carried out by mixing the samples with 6 mL of 65% nitric acid (HNO<sub>3</sub>) and 4 mL of 37% hydrochloric acid (HCl) to create a homogenized solution. Following digestion, the samples were diluted to a final volume of 100mL using a volumetric flask and filtered twice by using gravity filtration and microfiltration to remove any undigested particles for smooth analysis with AAS. The results of Fe contents in anchovies are 4.22 ppm (TF Mart), 5.16 ppm (Segi Fresh), 5.88 ppm (Pasar Mini Tapah) and 6.72 ppm (100 Mart) respectively. Meanwhile, for Cd the results are 0.06 ppm (TF Mart), 0.15 ppm (Segi Fresh), 0.15 ppm (Pasar Mini Tapah) and 0.41 ppm (100 Mart), respectively. The analysis shows that all HI values are below 1, indicating no significant health risks.

**Keywords:** Heavy metal pollution, Cadmium (Cd), Iron (Fe), Atomic absorption spectroscopy (AAS), Hazard index (HI)

## INTRODUCTION

Heavy metal pollution in aquatic environments is a significant concern due to industrial discharges, agricultural runoff, and urbanization. This pollution introduces harmful contaminants that adversely affect marine ecosystems and human health. One major issue associated with heavy metal pollution is bioaccumulation which occurs when these metals are absorbed by marine organisms at a faster rate than they can be metabolized or excreted. This accumulation, particularly in commercially important species like anchovies, poses risks to marine life and humans who consume contaminated seafood (Manev et al., 2021). Cd and Fe are of particular interest due to their toxicological implications and prevalence in marine environments. Cd is known for its high toxicity, even at low concentrations, and can cause severe health effects on marine species and humans. Cd primarily enters organisms through ingestion and inhalation (Ismail et al., 2018). Meanwhile, Fe, although essential for biological processes, can become harmful when present in excessive amounts, disrupting the delicate balance of aquatic ecosystems.

Despite advancements in research on heavy metal concentrations in marine organisms, significant gaps persist in understanding Cd and Fe levels in anchovies. Although various studies employing Atomic Absorption Spectroscopy (AAS) have documented heavy metal levels in fish tissues, comprehensive data on Cd and Fe concentrations in anchovies from Malaysia remain limited (Bat et al., 2014; Galaçchi et al., 2017). Furthermore, the Hazard Index (HI), a key measure of seafood safety based on heavy metal content, has not been thoroughly explored in anchovies from this region. These gaps hinder a complete understanding of the ecological and public health risks posed by heavy metal contamination through seafood consumption.

The objectives of this study are to determine the concentrations of Cd and Fe in anchovies using AAS and to assess the health risks associated with these metals by calculating their HI values. These anchovies are sourced from suppliers based in Pangkor Island, ensuring they represent the local fishing practices of the region. This study provides valuable insights into the assessment of heavy metal contamination in anchovies, contributing to the evaluation of environmental pollution and its implications on seafood safety. This research also underscores the importance of regular monitoring and regulatory measures to ensure anchovy products comply with safety standards.

## METHODOLOGY

### Sample Preparation

Random sampling was employed to select anchovy samples from four shops, ensuring coverage of major regions known for high anchovy distribution. Approximately 100 grams of anchovies were collected from each shop using a systematic random sampling method. The samples were dried in an oven at 60°C until a constant weight was

achieved. Once dried, they were ground into a fine, uniform powder using a laboratory blender to homogenize the sample and ensure consistency across analyses. Finally, the ground samples were sieved to achieve a consistent particle size for accurate digestion.

### Digestion Procedure & AAS Analysis

Approximately 1 g of ground anchovy sample was weighed into labelled beakers. In a fume hood, 6 mL of 65% HNO<sub>3</sub> and 4 mL of 37% HCl were added to each beaker. The samples were then heated on a hot plate (80–100°C) until the solution became clear. After cooling, each solution was transferred into a 100 mL volumetric flask and diluted to the mark with 1% HNO<sub>3</sub>. The diluted solutions were first filtered by gravity, followed by microfiltration for further purification. The final filtrates were analysed using Atomic Absorption Spectroscopy (AAS) (PerkinElmer Analyst 400) to determine the concentration of Fe and Cd.

### Data Analysis

The HI values for each shop can be determined based on the concentrations obtained from the AAS analysis. These concentrations are converted to mg/kg to calculate the Target Hazard Quotient (THQ) values. THQ is a number used to estimate the possible health risks of being exposed to a harmful substance, like heavy metals, from a certain source. It's often used to assess the risk of non-carcinogenic effects caused by contaminants found in food, water, or air. The formula to calculate THQ is:

$$THQ = (EF \times ED \times MS \times C / RfD \times BW \times AT) \times 10^{-3}$$

EF stands for exposure frequency, which is 365 days/year. ED is the exposure duration. The exposure duration is determined based on the average lifespan of Malaysians, which is approximately 70 years. MS represents the food ingestion rate, with Malaysians consuming fish daily at an average of 0.122 kg per day (Von Goh et al., 2021). C stands for concentration in mg/kg. RfD refers to the reference dose values for Fe and Cd, set at 0.70 mg/kg/day for Fe and 0.001 mg/kg/day for Cd, respectively (Karsli, 2021). BW stands for body weight, with Malaysians' average body weight is 62.65kg (Azmi, M. Y. et al., 2009). AT represents the average exposure time, calculated as 70 years (the average lifespan of Malaysians) multiplied by 365 days (the exposure frequency) (Pokorska-Niewiada et al., 2022).

HI is calculated to assess whether the anchovies from each shop are safe for consumption. The formula of HI is:

$$HI = THQ_{Fe} + THQ_{Cd}$$

This calculation is performed individually for each shop to assess the combined exposure to Fe and Cd from the anchovies available, ensuring a detailed and location-specific evaluation of potential risks.

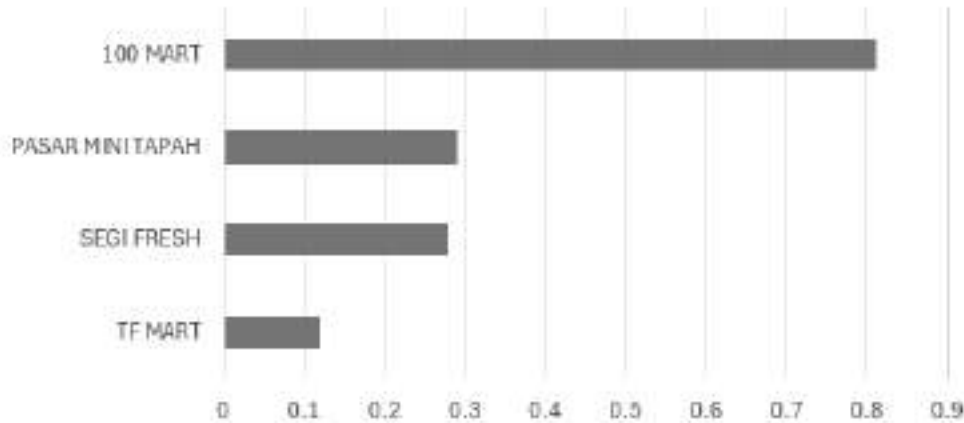
### FINDINGS

Table 1 presents the concentration levels of Fe and Cd along with their respective THQ and HI values, for anchovy samples obtained from four different shops. Concentration of Fe ranged between 4.22 mg/L (TF Mart) and 6.72 mg/L (100 Mart), with values converted to mg/kg ranging from 4033.26 mg/kg to 6617.43 mg/kg. Meanwhile, Cd concentrations were lower, ranging from 0.06 mg/L (TF Mart) to 0.41 mg/L (100 Mart), with corresponding values in mg/kg ranging from 57.34 mg/kg to 403.74 mg/kg.

**Table 1** Concentration, THQ and HI values for Fe and Cd in anchovies across four different shops

| Source           | Iron (Fe)    |               |      | Cadmium (Cd) |               |      | HI   |
|------------------|--------------|---------------|------|--------------|---------------|------|------|
|                  | Conc. (mg/L) | Conc. (mg/kg) | THQ  | Conc. (mg/L) | Conc. (mg/kg) | THQ  |      |
| TF Mart          | 4.22         | 4033.26       | 0.01 | 0.06         | 57.34         | 0.11 | 0.12 |
| Segi Fresh       | 5.16         | 4795.98       | 0.01 | 0.15         | 139.42        | 0.27 | 0.28 |
| Pasar Mini Tapah | 5.88         | 5524.24       | 0.02 | 0.15         | 140.92        | 0.27 | 0.29 |
| 100 Mart         | 6.72         | 6617.43       | 0.02 | 0.41         | 403.74        | 0.79 | 0.81 |

The calculated THQ values for Fe were consistently below 0.02, indicating minimal health risks. For Cd, the THQ was highest at 0.79 (100 Mart) but remained within acceptable safety limits. The combined HI values, representing the cumulative risk of Fe and Cd, remained below the critical threshold of 1 for all locations. The highest HI of 0.81 was recorded at 100 Mart, confirming that the anchovies were safe for consumption but underscoring the need for ongoing monitoring.



**Figure 1** HI values for anchovies in four different retail shops

The significant difference in HI values for anchovies from 100 Mart compared to other shops, despite all sources originating from Pulau Pangkor, suggests various possible factors influencing the contamination levels. One plausible explanation is post-harvest handling and storage practices. While the anchovies may be caught in the same waters, the way they are handled after being brought to shore can vary significantly between suppliers. Exposure to contaminated packaging materials or metal-based preservation techniques during storage at 100 Mart could have contributed to the higher cadmium concentration (Ahmed et al., 2020).

Another factor could be environmental or processing contamination. Even if all anchovies come from Pulau Pangkor, differences in drying methods or equipment used during processing could inadvertently introduce additional contaminants. For instance, using processing tools with metal components or operating in environments with exposure to pollutants may increase the cadmium levels in the anchovies supplied to 100 Mart (Wu et al., 2019).

Batch variability is also a likely contributor. Differences in harvesting times and specific fishing zones within Pulau Pangkor waters may result in variability in heavy metal accumulation. The batch supplied to 100 Mart may have come from an area with higher cadmium exposure due to localized contamination, possibly from industrial or agricultural runoff (Rahman et al., 2021).

Additionally, analytical or sampling inconsistencies might have influenced the results. Sampling methods that did not fully represent the overall stock at 100 Mart or cross-contamination during testing could lead to inflated cadmium levels. This highlights the importance of robust and standardized testing protocols to ensure reliable data (Zhang et al., 2020).

Finally, packaging materials may have played a role. If 100 Mart used non-food-grade packaging, metal leaching could occur, particularly in high-humidity or temperature environments, further elevating the cadmium content in the anchovies. These findings underscore the importance of investigating the entire supply chain, from post-harvest handling to packaging and distribution, to ensure consistent seafood safety even when sourced from the same location.

Overall, the study concludes that anchovies from the analyzed locations do not pose significant health risks based on the measured concentrations of Fe and Cd. However, continued monitoring and stricter environmental controls are recommended to address heavy metal pollution and ensure seafood safety. These findings contribute to the understanding of heavy metal bioaccumulation in anchovies and its implications for public health and environmental conservation.

## CONCLUSION

In conclusion, this study successfully determined the concentrations of Fe and Cd in anchovies from four shops in Tapah, Perak, using AAS. The results showed that all HI values were below the safety threshold of 1, indicating no significant health risks associated with the consumption of these anchovies. The highest HI value of 0.81 was recorded at 100 Mart, which is still within safe limits, highlighting the need for continuous monitoring. These findings confirm the study's objective of evaluating the safety of anchovies based on their heavy metal content and provide a clearer understanding of their quality and potential health impacts.

The findings underscore the importance of ensuring seafood safety and contribute to a deeper understanding of metal contamination in aquatic ecosystems. Future recommendations include broadening the scope of metal analysis, refining sampling strategies, and strengthening environmental policies to minimize contamination risks.

#### COMPLIANCE OF ETHICAL STANDARD

This study followed strict ethical and professional guidelines. All protocols were established to ensure the study's transparency, neutrality, and integrity. The funding sources for this study have been reported, and no potential conflicts of interest, financial or otherwise, have been detected. Ethical considerations for animal welfare were closely monitored, ensuring that the anchovies utilised in this study were sourced ethically and treated in accordance with recognised protocols. Any human involvement in sample collection or handling required informed consent, and participant privacy was protected. These indicators demonstrate a commitment to upholding high ethical standards throughout the study process.

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# INVESTIGATION OF FUEL CELL OUTPUT UNDER DIFFERENT ELECTROLYTE CONDITIONS AND RENEWABLE HYDROGEN GENERATION SOURCE FROM TELUK BATIK, MALAYSIA

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**Abstract:** Proton exchange membrane fuel cells (PEMFCs) represent a promising clean energy technology, providing an efficient and environmentally sustainable method of electricity generation through electrochemical reactions. The performance and efficiency of PEMFCs are strongly influenced by several parameters, including electrolyte type and concentration, operating temperature, input voltage, and the source of water used for electrolysis. Optimising PEMFC systems requires a comprehensive understanding of how these factors interact, particularly in relation to the application of non-conventional electrolytes and renewable water sources for hydrogen production. This study investigates the performance of PEMFCs under different electrolyte conditions (HCl and NaOH), renewable water sources such as seawater, and applied input voltages (5 V, 10 V, 15 V). The experimental design further examined the effects of different electrolytes with 0.5 M concentration and operating temperatures (room temperature, 70 °C, 80 °C, and 90 °C) on output voltage. Results demonstrate that electrolyte concentration, temperature, water source, and input voltage exert a significant influence on PEMFC efficiency and power output. Specifically, higher NaOH concentrations [0.5 M], elevated temperatures (90 °C), and greater applied voltages (15 V) enhanced hydrogen generation and overall cell performance. In contrast, the use of non-distilled water sources such as seawater was associated with reduced efficiency. These findings provide valuable insights into optimising PEMFC systems for sustainable energy production, particularly when integrating renewable water resources and non-traditional electrolytes.

**Keywords:** Proton exchange membrane fuel cells (PEMFC), Clean energy, Hydrogen production, Sea water, Electrolyte concentrations

## INTRODUCTION

The escalating cost of fossil fuels, combined with the imperative to mitigate greenhouse gas emissions, has intensified efforts to develop more efficient and environmentally sustainable energy generation technologies (Wang & Azam, 2024). Malaysia, endowed with extensive water resources and an average annual rainfall of approximately 2000 mm, offers considerable potential for renewable energy exploitation (Mekhilef, 2010). Rivers, lakes, and groundwater represent major freshwater reserves that can be strategically utilized for energy applications. A proton exchange membrane electrolyser (PEME), which generates clean hydrogen gas, operates in a complementary manner to a Proton Exchange Membrane Fuel Cell (PEMFC) for electricity production (Bodkhe, Shrivastava, Soni, & Chadge, 2023). This study employs Proton Exchange Membrane Fuel Cells (PEMFCs) and hydrogen fuel cells to generate energy from locally available water resources, including seawater, with research focused on the Teluk Batik shore in Perak, Malaysia. The main objective is to systematically evaluate the influence of four critical parameters—electrolyte type, electrolyte temperature, power input, and renewable water source such as seawater—on the performance, efficiency, and durability of hydrogen fuel cells. Electrolysis is an electrochemical process that utilizes electrical energy to dissociate water molecules into hydrogen (H<sub>2</sub>) and oxygen (O<sub>2</sub>) (Cavaliere, 2023). The efficiency of electrolysis is strongly dependent on several key parameters, including the type of electrolyte, operating temperature, applied power input, and the quality of the water source (Mazloomi & Sulaiman, 2012). Temperature exerts a significant influence on ion mobility and electrochemical reaction kinetics during the electrolysis process (Elder, Cumming, & Mogensen, 2015). The experimental scope includes the assessment of multiple electrolytes such as seawater, hydrochloric acid (HCl), and sodium hydroxide (NaOH) to understand their effects on output voltage, ionic conductivity, electrochemical reaction kinetics, and overall system efficiency. Investigations will be conducted with electrolyte concentrations [0.5 M], operating temperatures (ambient, 70 °C, 80 °C, 90 °C), and applied voltages (5 V, 10 V, 15 V).

The study will further examine the impact of power input on energy conversion efficiency, long-term stability, and operational durability. Additionally, the feasibility of employing unconventional water sources, such as seawater, will be explored with respect to their contribution to system performance. By analysing the interplay of these operating variables, this research seeks to establish synergistic conditions that maximize hydrogen fuel cell output and reliability. The outcomes are expected to validate the technical feasibility of optimized hydrogen fuel

cells for deployment in diverse environmental settings, thereby advancing the development of sustainable, cost-effective, and scalable hydrogen production technologies.

## **METHODOLOGY**

The experiment was designed to evaluate the effects of electrolyte concentration, temperature, and water source type on fuel cell performance. Hydrochloric acid (HCl), and sodium hydroxide (NaOH) were employed as electrolytes at concentration of 0.5 M. The PEMFC performance was assessed by measuring the voltage output at three voltage levels (5 V, 10 V, and 15 V) during a 1-minute test period using DC voltage. The 1-minute testing duration was selected because the output voltage continued to increase over time; thus, this time frame allowed any fluctuations or initial transient effects in the system to stabilize before measurement. The experiments were conducted at four distinct temperature settings: room temperature (23–24 °C), 70 °C, 80 °C and 90 °C, to examine the influence of temperature on the electrochemical reactions within the fuel cell. The study also included three seawater sources for electrolysis. The PEMFC system was connected to a power supply, and hydrogen production was recorded over a 1-minute interval for each combination of temperature and water source. For data collection, a digital millivoltmeter was used to measure the voltage output of the fuel cell, and the output was recorded at 1-minute intervals. Temperature control was maintained using thermal insulation, achieved by wrapping the electrolysis kit with a towel to minimize heat loss to the environment, thereby ensuring that the experimental conditions remained stable throughout the testing process. The data from each experimental run were subsequently analysed to determine the relationships among electrolyte type, concentration, temperature, and fuel cell performance, as well as the impact of renewable water sources on hydrogen generation.

### **Electrolyte Preparation**

#### **Preparation of HCl solutions**

To prepare 500 mL of 0.5 M HCl from a 12 M HCl stock solution, 20.83 mL of concentrated HCl was carefully measured using a pipette and transferred into a 500 mL volumetric flask containing approximately 200 mL of distilled water. The solution was mixed gently, allowed to cool, and then diluted to the calibration mark with distilled water to obtain the final 0.5 M HCl solution. The volumetric flasks were then labelled and set aside for experimental use.

#### **Preparation of NaOH solutions**

To prepare 500 mL of 0.5 M NaOH solution, 10 g of solid NaOH pellets was accurately weighed and dissolved in approximately 200 mL of distilled water in a beaker. The solution was stirred until fully dissolved and allowed to cool. It was then transferred into a 500 mL volumetric flask and diluted to the calibration mark with distilled water to obtain the final 0.5 M NaOH solution. The solutions were stirred until the NaOH pellets were completely dissolved, after which volumetric flasks were then labelled and set aside for experimental use.

#### **Filtration process for seawater**

The seawater water sample was filtered using filter paper to remove suspended particles and impurities. Each sample was poured through filter paper placed in a funnel until the water appeared clear and free of debris. A total of 1000 mL of each sample was collected and stored for subsequent experiments.

#### **Experiment setup**

The experiment was setup by the used of power supply with two wires connected to the PEM, two tubes connected to the hydrogen fuel cell for gas flow, and two additional wires connected to an ammeter for measuring the output voltage. To demonstrate the working principle of a hydrogen fuel cell, a small fuel cell kit was assembled, which included an anode, a cathode, and a proton exchange membrane that allowed only protons to pass through. A hydrogen source was generated by splitting water into hydrogen and oxygen through electrolysis, achieved by adding different electrolytes to enhance the conductivity of the water.

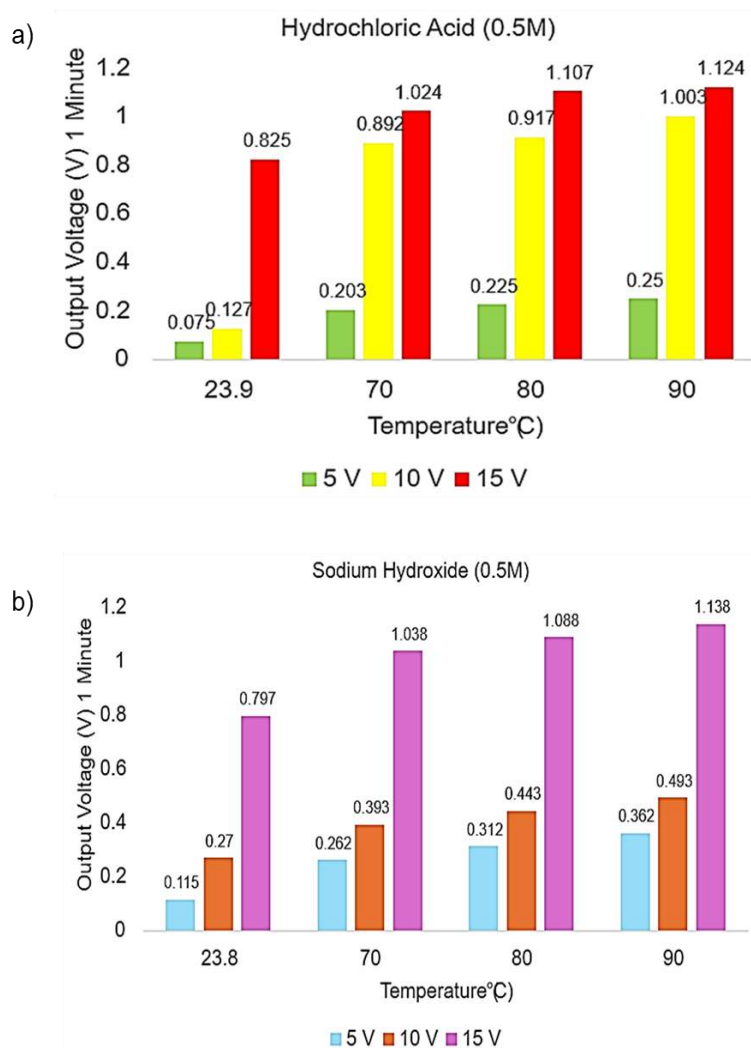
#### **Study Area and Site Selection**

Seawater sampling was performed at a shore site in Teluk Batik, Perak, Malaysia (4°11'19.4"N, 100°36'18.9"E).

## **FINDINGS**

The performance evaluation of hydrochloric acid (HCl) and sodium hydroxide (NaOH) as electrolytes revealed that higher concentrations and elevated temperatures markedly enhance electrolysis efficiency. Among the tested electrolytes, HCl and NaOH produced the highest output voltages, with NaOH at 0.5 M demonstrating the most consistent performance under all experimental conditions. In terms of alternative water sources, seawater shows potential as a renewable electrolyte for energy applications. The highest concentration, 0.5 M HCl, further improved electrolysis performance, yielding a maximum voltage of 1.124 V at 90 °C with 15 V applied, representing the optimum performance for HCl within the studied parameters. Comparable findings have been

reported that increasing analyte flow rate, concentration, oxygen flow rate, and temperature resulted in decreased cell voltage but increased current coulombic efficiency (CCE) (Joudaki, Mohammadi, Yousefi, Eivazkhani, & Yousefi, 2009).

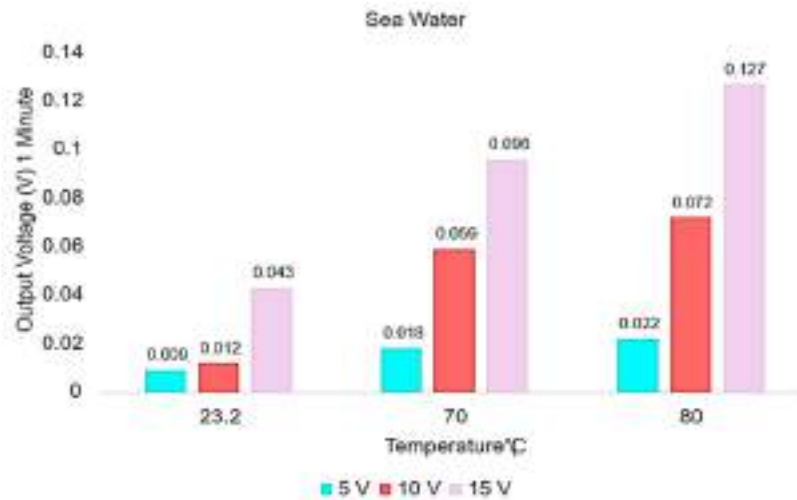


**Figure 1** (a) Temperature-dependent voltage output for HCl [0.5 M] at applied voltages of 5 V (green), 10 V (yellow), and 15 V (red). (b) Voltage–temperature relationship for NaOH [0.5 M] at applied voltages of 5 V (blue), 10 V (orange), and 15 V (purple).

The electrolysis performance of 0.5 M HCl shows a strong relationship between temperature, applied voltage, and output voltage in figure 1 (a). At room temperature (23.9 °C), the output voltages were relatively low, measuring 0.075 V, 0.127 V, and 0.825 V for applied inputs of 5 V, 10 V, and 15 V, respectively. As the operating temperature increased, output voltages improved markedly. At 70 °C, the recorded values rose to 0.203 V (5 V), 0.892 V (10 V), and 1.024 V (15 V). Further enhancement was observed at 80 °C, with outputs of 0.225 V, 0.917 V, and 1.107 V for 5 V, 10 V, and 15 V, respectively. The highest performance occurred at 90 °C, yielding maximum voltages of 0.250 V (5 V), 1.003 V (10 V), and 1.124 V (15 V). These results confirm that higher electrolyte concentration, in combination with elevated temperature and applied voltage, substantially enhances electrolysis efficiency (Zhang et al., 2023).

The electrolysis performance of 0.5 M NaOH exhibits a consistent increase in output voltage with rising temperature and applied voltage as shown in figure 1 (b). At room temperature (23.8 °C), the recorded values were 0.115 V, 0.270 V, and 0.797 V for inputs of 5 V, 10 V, and 15 V, respectively. At 70 °C, the output increased to 0.262 V (5 V), 0.393 V (10 V), and 1.038 V (15 V). Further improvements were observed at 80 °C, yielding 0.312 V, 0.443 V, and 1.088 V, while the highest performance was achieved at 90 °C, with maximum voltages of 0.362 V (5 V), 0.493 V (10 V), and 1.138 V (15 V). The electrolysis performance of NaOH demonstrates a pronounced enhancement in output voltage with increasing concentration and temperature. The highest concentration tested, 0.5 M NaOH yielded the best performance, achieving 1.138 V at 90 °C and 15 V, representing the peak electrolysis efficiency across all NaOH concentrations. These results confirm that higher

NaOH concentration, coupled with elevated temperature and applied voltage, delivers the strongest electrolysis efficiency among the tested conditions (Zhang et al., 2023).



**Figure 2** Temperature-dependent voltage output of seawater (boiling point: 97.4 °C) at applied voltages of 5 V (blue), 10 V (red), and 15 V (light purple).

The influence of renewable energy sources, such as solar and wind, on electrolysis systems can be assessed through the performance of alternative electrolytes and natural water samples. The electrolysis behaviour of natural waters demonstrates considerable variability. Hydrogen production via the electrolytic decomposition of water is scientifically straightforward, reliable, and clean, with fundamental principles established in the early 19<sup>th</sup> century (Fickett & Kalhammer, 2018). However, large-scale adoption has been limited by the high cost of electricity relative to fossil-based feedstocks such as natural gas or naphtha (Zanon-Zotin et al., 2024).

Since electrolysis plant design is minimally affected by oxygen utilization—and on-site oxygen applications remain rare—hydrogen is the primary focus of water electrolysis studies. Advances in electrolysis technologies aim to reduce these limitations, bringing practical hydrogen production closer to its theoretical efficiency limits. Moreover, figure 2 for seawater (boiling point 97.4 °C) at Teluk Batik shore, Perak indicates a progressive increase in output voltage with both temperature and input voltage. At room temperature (23.2 °C), the output voltage is recorded as 0.009 V at 5 V, 0.012 V at 10 V, and 0.043 V at 15 V. When the temperature is raised to 70 °C, the output voltage increases to 0.018 V (5 V), 0.059 V (10 V), and 0.096 V (15 V). At 80 °C, the output improves further, reaching 0.022 V (5 V), 0.072 V (10 V), and 0.127 V (15 V). Seawater (boiling point 97.4 °C) exhibits encouraging performance, with output voltage gradually increasing alongside temperature and applied voltage, reaching a maximum of 0.127 V at 80 °C with a 15 V input as shown in figure 2. Compared to a pure electrolyte, the dissolved salts in seawater decrease the electrolytic efficiency; therefore, pretreatment is necessary to enhance the output voltage (Shetty et al., 2025).

## CONCLUSION

The findings of this study provided valuable insights into the performance of Proton Exchange Membrane Fuel Cells (PEMFCs) under varying electrolyte types, concentrations, temperatures, and water source conditions. The results demonstrated that both temperature and electrolyte concentration exerted a significant influence on output voltage and electrolysis efficiency. Among the tested electrolytes, NaOH at 0.5 M consistently produced the highest output voltages, with optimal performance achieved at elevated temperatures (90 °C) and higher applied voltages (15 V). For alternative water sources, seawater collected at a nearshore site Teluk Batik, Perak displayed notable improvements in output voltage with increasing temperature and applied voltage, suggesting its potential as a practical electrolyte for renewable energy applications. Despite experimental limitations, such as challenges in temperature control and equipment precision, the results underscore the importance of water quality, electrolyte concentration, and temperature in enhancing hydrogen production for PEMFC applications. These findings indicate that seawater electrolysis offers a viable pathway to support the advancement of a sustainable hydrogen economy, with potential implications for clean transportation, energy storage, and emission reduction across multiple sectors. Future research should focus on improving seawater-based electrolysis efficiency and addressing current experimental constraints. Key directions include investigating the influence of seawater composition from different geographical locations other than shore at Teluk Batik, Perak on output voltage, exploring alternative electrode materials to enhance reaction kinetics, and examining the role of catalysts in increasing electrolysis efficiency. Additionally, microbial activity within seawater may impact the electrolysis process; thus, identifying

native microorganisms and developing strategies to mitigate their effects could further optimize system performance. Addressing these areas of research will be critical for advancing seawater-based hydrogen production and accelerating the transition toward a sustainable hydrogen economy.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

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# FORMULATION AND CHARACTERIZATION OF BAR, PASTE, AND LIQUID SOAPS USING RECYCLED COOKING OIL

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**Abstract:** This research focuses on the development of soaps formulated from the recycled cooking oil as the primary raw material. The objective was to produce soaps capable of effectively reducing bacterial presence, suitable for all skin types, and available in three distinct forms: solid, paste, and liquid. The formulations were prepared by combining sodium hydroxide, distilled water, and repurposed cooking oil, with lime juice incorporated for its antibacterial properties. The physicochemical evaluations were conducted to examine parameters such as pH stability, foaming capacity, allergenic potential, and colour. The results show the potential of upcycling waste materials into consumer-friendly soap products.

**Keywords:** *Paste soap, Liquid soap, Solid soap, Saponification, Used cooking oil*

## INTRODUCTION

The increasing global focus on sustainability and consumer demand for multifunctional personal care products has driven innovation in soap production (Martins, 2020; WHO, 2020). While conventional soaps are typically produced using fresh oils and fats, recycled cooking oil has emerged as a sustainable and cost-effective alternative that reduces environmental waste (Okpo & Edafiaadhe, 2024). Soap remains one of the most essential hygiene products due to its ability to emulsify oils, dirt, and microbes through the saponification process, where triglycerides are converted into soap and glycerol using alkalis such as sodium hydroxide (Benedict, 2024). Beyond its cleansing properties, soap also plays a historical and cultural role in daily hygiene, underlining its universal relevance (Zahran, 2023)

Research demonstrates that incorporating renewable, biodegradable, and non-toxic ingredients results in environmentally safe soaps with desirable physicochemical properties. Comparative studies further reveal that antibacterial soaps do not always outperform regular soaps in reducing bacteria, reinforcing the effectiveness of simple, well-formulated natural soaps (Kanyama, 2025).

In this study, recycled cooking oil was employed as the primary base material, combined with sodium hydroxide as the alkali, lime juice for antibacterial enhancement, and lavender fragrance for the aroma. Soaps were prepared in solid, paste, and liquid forms and tested for pH stability, foaming capacity, colour, and allergenicity. The results demonstrated that soaps made from recycled cooking oil exhibited antibacterial properties, stable pH levels, enhanced foaming ability, and safety for human use, highlighting their potential as sustainable, biodegradable, and consumer-friendly hygiene products.

Previous research has examined the influence of different oils on soap properties, with findings showing that solid soaps provide greater hardness and durability, while liquid soaps excel in lathering and moisturizing qualities (Arasaretnam & Venujah, 2019). From an environmental perspective, the use of recycled cooking oil in soap production significantly reduces carbon footprint and waste generation compared to conventional. Research is still limited on the effects of soap ingredients on skin conditions, particularly for consumers seeking antibacterial formulations that support skin health, such as eczema treatment.

There is also a lack of standardized methods to ensure consistency across different batches of soap made from recycled cooking oil. Furthermore, little is known about the long-term performance and shelf life of such products in terms of consumer satisfaction and stability. This study contributes to addressing these gaps by demonstrating the potential of recycled cooking oil in producing safe, effective, and eco-friendly soaps, thereby supporting sustainable waste management practices and offering viable alternatives to conventional soap manufacturing.

## METHODOLOGY

### Preparation of the Soap

Soaps were produced in three forms: solid, paste, and liquid. For solid soap, 24 grams of sodium hydroxide

(NaOH) were weighed and dissolved in distilled water. The solution was handled carefully, as it became hot during the reaction. Then, 300 grams of cooking oil were slowly added while the mixture was blended using a hand blender until it formed a homogeneous solution. Approximately 15 ml of lime juice were added to enhance the antibacterial properties and aroma of the soap. The mixture was then poured into molds and left at room temperature for one week to harden. For paste and liquid soaps, from the same mixture, it was dissolved in hot distilled water to diluted. The mixture was stirred until a gel-like paste formed. One portion was retained as paste soap, while the other was diluted with additional distilled water to produce a liquid form. Lavender fragrance was added to improve the scent of the soap. The soaps were then left to cured for four weeks.

### pH Testing

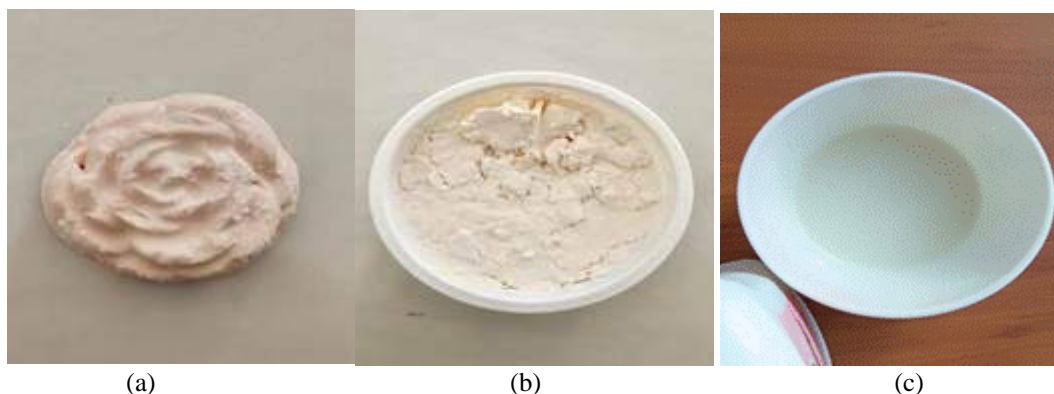
The pH of the soaps was measured using a pH meter. Samples were taken during production to confirm complete saponification and to prevent excessive alkalinity or acidity.

### Physicochemical Testing

Physicochemical properties have been evaluated including colour, foaming ability, pH stability, and potential allergenicity. Feedback was also collected from student users to assess the performance and safety of the soaps.

### FINDINGS

Three soap forms were successfully produced using the waste cooking oil is feasible, with three distinct forms liquid, paste, and solid. It's been produced through the saponification process. Figure 1 illustrates the soap products made from waste cooking oil, namely: S.B (Soap Bar), S.P (Soap Paste), and S.L (Soap Liquid). The pH level was identified as a crucial parameter for evaluating soap quality, cleaning effectiveness, and compatibility with human skin. Since natural soaps typically have a pH between 9–10, which balances cleansing with skin comfort (Anantharaman et al., 2018; Tarun et al., 2020), the measured values of 12.18 for liquid soap, 11.54 for paste soap, and 11.50 for solid soap indicate higher alkalinity.



**Figure 1** Illustrates the soap products: (a) S.B – Soap Bar, (b) S.P – Soap Paste, and (c) S.L – Soap Liquid

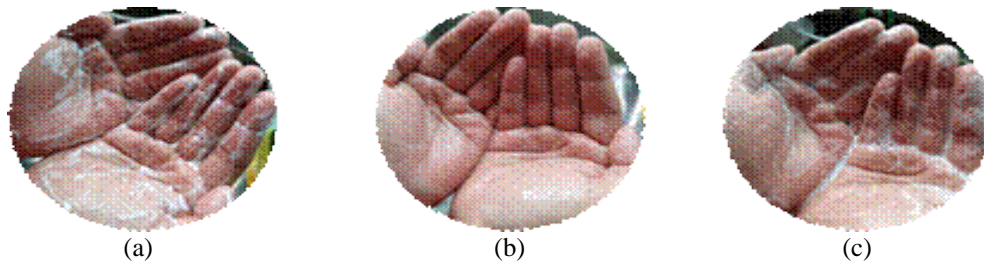
This survey was conducted to gather valuable feedback on the performance and quality of soaps made from used cooking oil in three forms: liquid, paste, and solid. A physicochemical testing form was used to evaluate parameters such as texture, effectiveness, scent, and overall user experience. This feedback was critical in identifying areas for improvement in the formulations, with the goal of developing soaps that are safe for skin, effective in cleaning, and acceptable to consumers. The study successfully demonstrated that soap production from used cooking oil through saponification with sodium hydroxide is feasible, yielding three distinct product types.

**Table 1** shows the physicochemical testing for the Soap Bar (S.B), Soap Paste (S.P), and Soap Liquid (S.L).

| Type of cooking oil soap | Physical test |           |               |       |
|--------------------------|---------------|-----------|---------------|-------|
|                          | Color         | Foam test | Allergic test | pH    |
| Soap Bar (S.B)           | Milky white   | Low       | negative      | 11.50 |
| Soap Paste (S.P)         | Milky White   | High      | negative      | 11.54 |
| Soap Liquid (S.L).       | Milky white   | Medium    | negative      | 12.18 |

Table 1 show the physicochemical testing for the Soap Bar (S.B), Soap Paste (S.P), and Soap Liquid (S.L). The results indicated that pH level is an essential factor in assessing soap quality, cleansing efficiency, and skin

compatibility. Typically, natural soaps fall within the pH range of 9–10, balancing cleaning effectiveness with skin comfort. In this study, however, all three soaps showed higher alkalinity, with recorded pH values of 12.18 for liquid soap, 11.54 for paste soap, and 11.50 for solid soap. These values exceeded the standard range, suggesting potential challenges for safe skin application.



**Figure 2** The level of soapiness on hands for the three soap types: (a) S.B – Soap Bar, (b) S.P – Soap Paste, and (c) S.L – Soap Liquid.

The liquid soap, formulated with lavender fragrance, had the highest pH (12.18), making it the most alkaline of the three. While this high alkalinity enhanced its cleaning strength, it also raised concerns regarding possible skin irritation. This elevated pH could be due to incomplete neutralization of sodium hydroxide or insufficient dilution during the saponification process. Additionally, the type of oil used influenced foam production. Oils high in unsaturated fatty acids, such as palm oil, generally produce less foam than oils with higher saturated fatty acid content, such as coconut oil (Arasaretnam & Venujah, 2019).

The paste soap had a slightly lower pH of 11.54 but remained strongly alkaline. Its thick and concentrated texture made it effective for cleaning tough grease and dirt. However, it tended to leave sticky residues on the skin due to its low solubility. The high surfactant content and low water level helped generate stable foam, but also increased the risk of skin dryness or irritation. The solid soap, which included lime juice, showed the lowest pH at 11.50. The lime juice slightly reduced the alkalinity, making it gentler on the skin compared to the other types.



**Figure 3** Cleaning effectiveness of S.B (Soap Bar), S.P (Soap Paste), and S.L (Soap Liquid) on plates before, during, and after washing

Figure 3 illustrates the cleaning effectiveness of the three soap types—S.B (Soap Bar), S.P (Soap Paste), and S.L (Soap Liquid)—showing the condition of a dirty plate before washing, during cleaning, and after cleaning, where all three soaps produced comparable results. The findings confirmed that soaps produced from used cooking oil are effective cleaning agents and represent a sustainable alternative to conventional formulations. However, the elevated pH values observed across all types highlighted limitations in skin compatibility.

## CONCLUSIONS

This study confirms that the saponification of used cooking oil provides a sustainable method for producing soaps in liquid, paste, and solid forms, demonstrating both environmental and economic value by transforming waste into useful hygiene products. Future improvements should be done to focus on refining the neutralization process, adjusting dilution ratios, and incorporating buffering agents or natural additives to achieve a milder pH. These modifications will enhance consumer safety and comfort, ensuring that the soaps remain both environmentally sustainable and user-friendly.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

## ACKNOWLEDGEMENT

The authors sincerely thank the experts in Faculty of Applied Sciences, Universiti Teknologi MARA, Perak

Branch Tapah Campus for commitment and contribution.

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# DEVELOPMENT AND EVALUATION OF ROSELLE SOAP USING PALM AND COCONUT OILS

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**Abstract:** This study focuses on the formulation of natural soap enriched with roselle (*Hibiscus sabdariffa*) extract using two different base oils: palm oil and virgin coconut oil. Roselle is known for its antioxidant and antibacterial properties, making it a promising natural ingredient for skincare. The objective was to compare the physical and functional qualities of palm oil-based and coconut oil-based soaps, with emphasis on texture, lather, color, and cleansing performance. Soap samples were prepared using the cold process method and tested for cleansing efficiency using marker stain removal time. Findings indicate that coconut oil-based soap produced harder bars with rich, bubbly lather, while palm oil-based soap created softer bars with creamy, stable lather. The palm oil formulation showed faster cleansing (12.76 s) compared to coconut oil (15.63 s), though the latter maintained roselle's reddish-brown color more effectively. This study highlights roselle's potential as a functional and sustainable additive in natural soap production, aligning with growing consumer demand for eco-friendly personal care products.

**Keywords:** *Roselle, Hibiscus sabdariffa, Soap, Palm oils, Coconut oils*

## INTRODUCTION

Growing consumer demand for natural skincare products has encouraged the exploration of plant-based ingredients with functional benefits. Roselle (*Hibiscus sabdariffa*), a tropical plant rich in anthocyanins, flavonoids, and organic acids, has been studied for its antioxidant, antibacterial, and anti-inflammatory properties (Cavalcanti et al., 2020; Riaz et al., 2021). These compounds contribute to reducing oxidative stress and enhancing skin health.

Several herbal soaps have been developed using turmeric (Monisha, 2021), green tea (Crudu, 2024), and papaya (Heena & Sunil, 2019), demonstrating the potential of plant extracts in improving cosmetic functionality. However, limited studies have examined roselle in soap formulations, especially regarding its stability during saponification and its topical effectiveness.

Palm oil and coconut oil are among the most common soap base oils. Coconut oil produces a harder soap with strong cleansing but can be drying, while palm oil contributes to creamier texture and milder cleansing (Dayrit, 2014; Botanie Soap, 2025). Combining these oils with roselle extract provides an opportunity to develop a soap with enhanced cleansing, aesthetic qualities, and skin benefits.

Therefore, this study aims to formulate roselle soap using palm and coconut oils and evaluate their performance through comparative analysis of texture, lather, color, and cleansing ability.

## METHODOLOGY

This research employed the cold process method to prepare Roselle soap. Fresh Roselle calyces were cut into small pieces and boiled in distilled water to extract pigments and bioactive compounds. The extract was then filtered and combined with measured quantities of palm oil or virgin coconut oil. A lye solution, prepared from sodium hydroxide, was added to each mixture. The solutions were mixed with a hand blender until homogeneous, poured into soap molds, and allowed to cure for 24 to 48 hours.

The cleansing ability was assessed using a marker stain removal test. A square mark was drawn on a volunteer's hand, which was then washed with each soap formulation separately. The time taken for complete removal of the stain was recorded. In addition to cleansing, other qualitative observations were made regarding soap texture, lather quality, and retention of Roselle's natural pigment. These parameters provided a comprehensive evaluation of how different oils influenced Roselle soap properties.

## FINDINGS

The virgin coconut oil-based Roselle soap produced a harder bar with a reddish-brown hue that reflected greater pigment retention. Its lather was rich, bubbly, and fluffy, consistent with previous reports that coconut oil produces abundant lather due to its lauric acid content (Dayrit, 2014). However, the cleansing test revealed that it took 15.63 seconds to remove the marker stain, making it slightly less efficient compared to palm oil-based soap. While its durability was advantageous, the soap's tendency to be drying on the skin suggests that coconut oil formulations require balancing with conditioning agents.

**Table 1** Comparison of roselle soap properties

| Aspect            | Coconut Oil-Based Soap | Palm Oil-Based Soap         |
|-------------------|------------------------|-----------------------------|
| Texture           | Harder bar             | Softer, brittle bar         |
| Color             | Reddish-brown retained | White, muted color          |
| Lather quality    | Rich, bubbly, fluffy   | Creamy, stable, less bubbly |
| Cleansing ability | 15.93 s                | 12.76 s                     |

Palm oil-based Roselle soap, in contrast, exhibited a softer texture and produced a creamier, more stable lather. The soap's whitish color indicated muted retention of Roselle pigments, likely due to palm oil's creamy base diluting the extract's natural hue. However, the cleansing test showed superior performance, removing the stain in 12.76 seconds. This supports previous findings that palm oil provides balanced cleansing and moisturizing properties (Botanie Soap, 2025). The refatting quality of palm oil, which helps restore skin lipids, contributed to its gentler feel on the skin, making it more suitable for individuals with sensitive skin.

Overall, the results demonstrate that both oils confer distinct advantages. Coconut oil-based soap excels in hardness and foaming ability, making it durable and visually appealing with a natural reddish tint. Palm oil-based soap, meanwhile, excels in moisturizing capacity and cleansing efficiency, though at the expense of color retention. These findings align with studies on other plant-based soaps, which emphasize the influence of base oils on product characteristics (Arasaretnam & Venujah, 2019).

The incorporation of Roselle further enhances both formulations by contributing antioxidants and antibacterial compounds. This indicates that Roselle-infused soaps not only meet basic cleansing requirements but also provide additional skin benefits, aligning with consumer demand for multifunctional natural skincare products.

## CONCLUSIONS

The study demonstrated that Roselle is a valuable additive in natural soap formulations, offering both functional and aesthetic benefits. Coconut oil-based Roselle soap produced harder bars with rich lather and color retention but required longer time for cleansing. Palm oil-based Roselle soap, by contrast, provided creamier lather, gentler cleansing, and faster stain removal, though its texture was softer and color less vibrant. These differences underline the importance of oil selection in soap-making.

For future improvements, stability testing should be conducted to assess soap durability over time. Incorporating natural hardeners such as beeswax or cocoa butter could improve texture, while the use of natural pigments like beetroot powder may enhance color stability. Expanding research into consumer acceptability and dermatological testing will also provide further insights into the commercial viability of Roselle soap. Ultimately, Roselle soap offers a sustainable, eco-friendly, and health-conscious alternative to commercial soaps, aligning with global trends toward natural skincare solutions.

## COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

## ACKNOWLEDGEMENT

The authors sincerely thank the experts in Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch Tapah Campus for commitment and contribution.

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# QUANTITATIVE ASSESSMENT OF HEAVY METAL CONTAMINATION IN LAKE WATER USING ATOMIC ABSORPTION SPECTROMETRY

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**Abstract:** This study investigated the concentrations of cadmium (Cd), chromium (Cr), and nickel (Ni) in two lakes and evaluated their compliance with the Malaysian National Water Quality Standards (NWQS). The analytical procedure was validated using calibration curves with good linearity ( $R^2 = 0.984\text{--}0.9949$ ). The results revealed severe heavy metal contamination, with all metals exceeding permissible limits. Cadmium concentrations (0.34–0.40 ppm) and nickel concentrations (1.52–1.53 ppm) showed the most dramatic exceedances, surpassing their regulatory thresholds (0.01 ppm and 0.05 ppm, respectively) by more than an order of magnitude. Chromium levels (0.1–0.3 ppm) also consistently breached the safety limit (0.05 ppm), with concentrations 2 to 6 times higher than allowed. The findings confirm a critical level of pollution, with the notably high concentrations of Cd and Ni being of particular concern due to their chronic toxicity and bioaccumulation potential, indicating a substantial risk to aquatic ecosystem integrity and public health.

**Keywords:** *Heavy metal, Water pollution, Lake water, Bioaccumulation*

## INTRODUCTION

Freshwater ecosystems are vital for sustaining global biodiversity, human health, and socioeconomic development. However, these critical resources face increasing threats from anthropogenic activities, including rapid urbanization, industrial discharge, and agricultural runoff, leading to widespread contamination by heavy metals (Islam et al., 2015). Among these pollutants, cadmium (Cd), chromium (Cr), and nickel (Ni) are particularly concerning due to their environmental persistence, toxicity, and capacity to bioaccumulate in aquatic food webs (Ali et al., 2019). The introduction of these metals into freshwater systems poses significant long-term risks to ecological integrity and public health.

In Malaysia, the protection of water resources is guided by the National Water Quality Standards (NWQS), which set permissible limits for pollutants in drinking and raw water. Nevertheless, consistent findings of heavy metal contamination in rivers, lakes, and reservoirs highlight ongoing challenges in pollution control, often linked to industrial effluents, untreated wastewater, and land-use changes (Al-Badaii et al., 2022; Yap et al., 2021). Exposure to elevated levels of Cd, Cr, and Ni is associated with serious health outcomes, including nephrotoxicity, carcinogenesis, and neurotoxic effects, underscoring the need for stringent monitoring (Genchi et al., 2020).

Recent studies in Malaysia continue to document heavy-metal contamination in inland water bodies. For instance, Ahmed et al. (2020) reported persistent cadmium and chromium along the Langat River supply chain, even after water treatment. In Sabah, Rahim et al. (2024) found elevated levels of chromium and nickel in both water and sediment of the Lohan River. Bioindicator research also supports these concerns; Jumaat & Ab Hamid (2023) demonstrated bioaccumulation of cadmium in damselfly larvae from Malaysian rivers. Moreover, studies of wastewater effluents reveal that nickel and cadmium remain prevalent in treated sewage plant discharges, indicating potential sources of freshwater contamination.

Advances in analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma optical emission spectroscopy (ICP-OES) have significantly enhanced the detection of trace metals, offering high sensitivity and multi-element capability (Beauchemin, 2022). However, in many environmental laboratories, particularly in developing regions, flame atomic absorption spectroscopy (FAAS) remains the method of choice due to its affordability, operational simplicity, and instrument availability, despite its lower sensitivity compared to more advanced techniques. When properly calibrated, FAAS continues to provide reliable and accurate quantification for routine monitoring of heavy metals in water samples.

This study aims to determine the concentrations of cadmium, chromium, and nickel in two selected lakes using FAAS and evaluate their compliance with the Malaysian NWQS. By quantifying the extent of heavy metal pollution and assessing its regulatory implications, this research provides essential baseline data to inform management strategies, support regulatory enforcement, and protect both aquatic ecosystems and public health.

## METHODOLOGY

### Chemicals and instrumentation

All reagents and solvents used were of analytical grade or HPLC grade. Nitric acid (HNO<sub>3</sub>) was purchased from Fisher Scientific. Standard solutions of chromium, nickel and cadmium were purchased from Merck KGaA, Darmstadt, Germany. Deionized water was used for analysis. Perkin Elmer Atomic Absorption Spectrometer AAnalyst 700 equipped with a deuterium background corrector and WinLab32 software was used for analysis.

### Preparation of stock solutions

Stock solutions of chromium (Cr), cadmium (Cd), and nickel (Ni) were prepared at a concentration of 1000 ppm in 250 mL volumetric flasks using deionized water. Working standard solutions (0.2–5 ppm) were subsequently prepared by serial dilution of the respective stock solutions.

### Sampling and pre-treatment of samples

Water sampling was conducted at three locations within Lake Alpha and Lake Zeta near UiTM Tapah Campus, Perak, Malaysia. At each site, samples were gathered from a depth of 10–15 cm below the surface using pre-cleaned, acid-washed 500 mL high-density polyethylene (HDPE) bottles. To remove suspended particulates, the samples were filtered through 0.45 µm Millipore filters. All samples were then transported on ice and refrigerated at 4°C until laboratory analysis.

### Water samples preparation

A 100 mL aliquot of an acid-preserved water sample was subjected to acid digestion. The sample was transferred to a 250 mL Erlenmeyer flask, mixed with 5 mL of concentrated HNO<sub>3</sub>, and covered with a watch glass. The mixture was heated slowly on a hot plate with the aid of boiling chips and evaporated until the volume was reduced to 15–20 mL. Subsequently, 10 mL of concentrated HNO<sub>3</sub> was added to complete the digestion. After cooling, the digestate was diluted with 50 mL of deionized water and re-heated to boiling to expel nitrogen oxides. Finally, the sample was filtered, made up to a final volume of 100 mL with deionized water, and mixed thoroughly.

### Method validation

The concentrations of heavy metals were determined using an external calibration method. A series of calibration standards were prepared from a 100-ppm stock solution. The calibration ranges were 1–5 ppm for Cr, 0.2–1.0 ppm for Cd, and 0.5–4.0 ppm for Ni. Linearity of the analytical response was established by performing a linear regression analysis of the peak area versus concentration for each metal.

## FINDINGS

The calibration curves for all metals exhibited good linearity, with correlation coefficients (R<sup>2</sup>) ranging from 0.984 to 0.9949 (Figure 1).

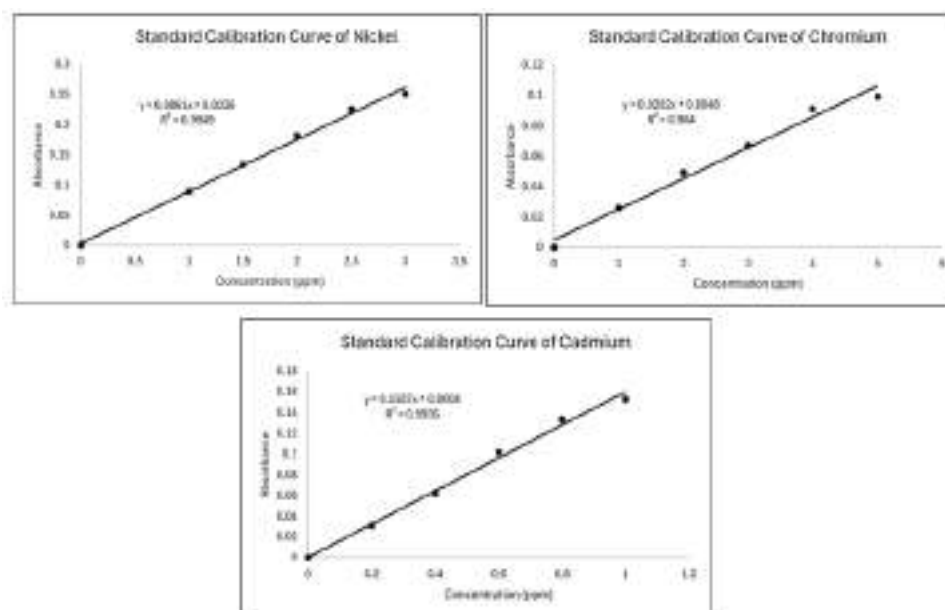
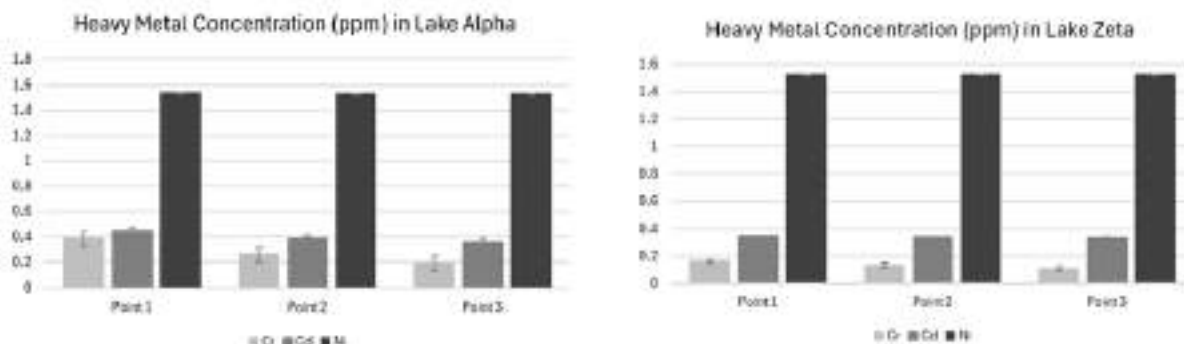


Figure 1 Standard calibration curve for Cd, Cr and Ni

### Heavy Metal Concentrations

The concentrations of heavy metals in Lakes Alpha and Zeta are presented in Figure 2, respectively. In both lakes, nickel was the most prevalent metal, with concentrations ranging from 1.52 to 1.53 ppm. This was followed by cadmium (0.34–0.40 ppm), while chromium exhibited the lowest concentrations (0.1–0.3 ppm). The consistent trend across all sampling points highlights nickel as the dominant contaminant in these ecosystems.



**Figure 2** Heavy metals concentration in both lakes

The measured concentrations of heavy metals were compared against the National Water Quality Standards for Malaysia (NWQS) by the Department of Environment Malaysia (DOE). The maximum permissible levels (MPL) of Cr, Cd and Ni in drinking water (or raw water criteria) is presented in Table 1.

**Table 1** National Water Quality Standards for Malaysia (NWQS) in drinking water (or raw water criteria)

| Metal    | Maximum permissible levels (ppm) |
|----------|----------------------------------|
| Cadmium  | 0.01                             |
| Chromium | 0.05                             |
| Nickel   | 0.05                             |

The analysis revealed severe heavy metal contamination in both lakes, with all measured metals exceeding national safety standards. The most dramatic exceedances were for cadmium (0.34–0.40 ppm) and nickel (1.52–1.53 ppm), which surpassed their permissible limits (0.01 and 0.05 ppm, respectively) by more than an order of magnitude. Chromium levels (0.1–0.3 ppm) also consistently breached the regulatory threshold (0.05 ppm), with concentrations 2 to 6 times higher than allowed. The elevated levels of Cd and Ni are of particular concern due to their well-documented chronic toxicity and potential for bioaccumulation in the food chain.

### CONCLUSIONS

In conclusion, this study confirms severe heavy-metal contamination in the studied lakes, with levels of Cd, Ni, and Cr exceeding regulatory limits. The extreme exceedance of Cd and Ni is of particular concern due to their well-documented chronic toxicity and bioaccumulation potential. These findings underscore the urgent need for implementing effective pollution-control measures, continuous monitoring, and remediation strategies.

To mitigate these risks, future work should focus on identifying the specific pollution sources, evaluating bioaccumulation in the aquatic food web, and modeling long-term ecological risks. Strengthening regulatory enforcement and improving wastewater management are essential steps to safeguard both aquatic ecosystems and public health. This study provides critical baseline data that emphasizes the necessity of protecting freshwater resources from persistent toxic contaminants.

### COMPLIANCE OF ETHICAL STANDARDS

*Not applicable.*

### ACKNOWLEDGEMENT

The authors sincerely thank the experts in Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch Tapah Campus for commitment and contribution.

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