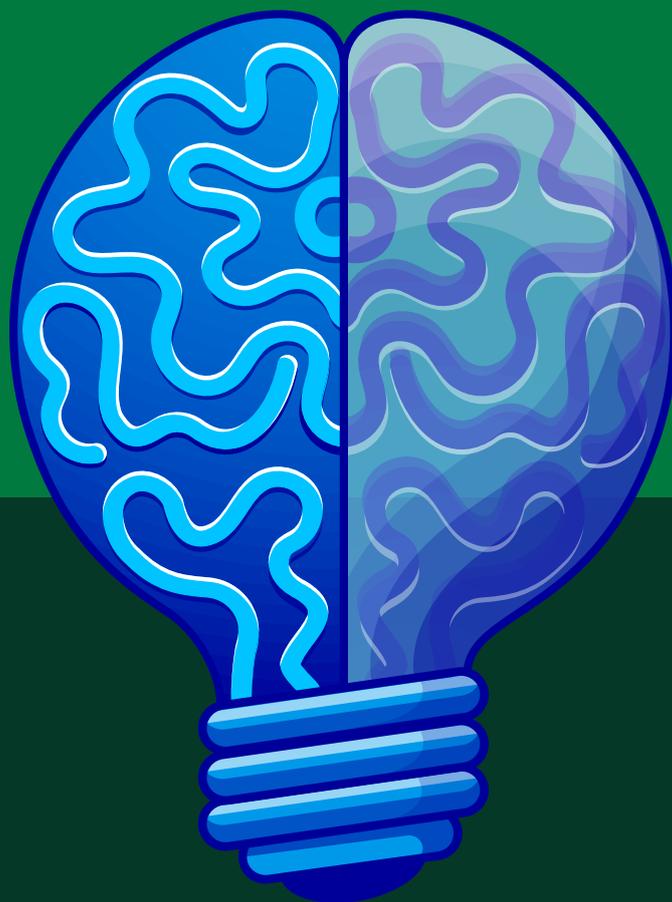


FACULTY OF  
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UNIVERSITI TEKNOLOGI MARA  
PERAK BRANCH

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BIOLOGY ~ CHEMISTRY ~ PHYSICS

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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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## 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**

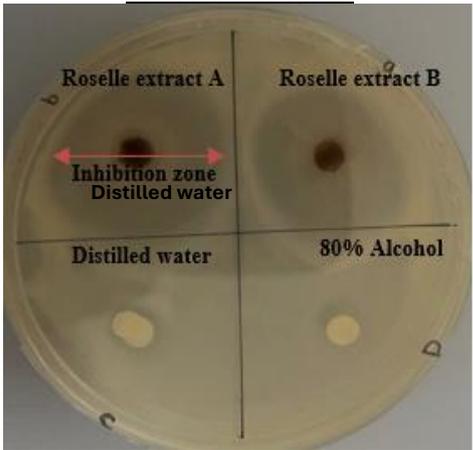
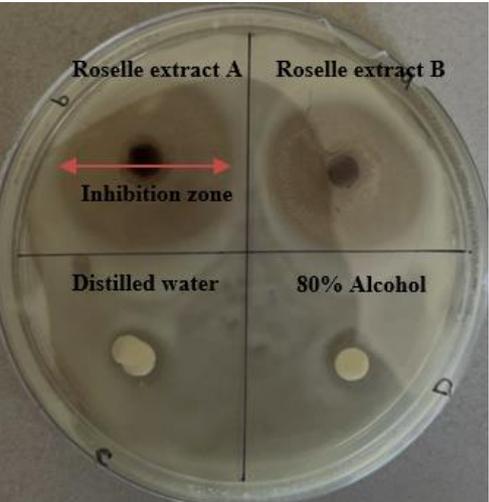
Four sections were drawn on the back of the agar petri dish using a marker pen, labelled as follows: A duplicates sample of roselle extract (roselle extract A and roselle extract B), C for distilled water (negative control) alcohol (positive control), and D for alcohol (positive control). A 200 µL volume 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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Tarikh : 20 Januari 2023

Prof. Madya Dr. Nur Hisham Ibrahim  
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Sekian, terima kasih.

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Saya yang menjalankan amanah,

*Setuju.*

*27.1.2023*

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