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Universiti Teknologi MARA,
Perak Branch Tapah Campus,
35400 Tapah Road, Perak, Malaysia.

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EDITORS
Pn. Rosliza Ali
Pn. Nunshaimah Salleh
Pn. Norsakina Zurina Zulkifli
Pn. Adibatul Husna Fadzil
Pn. Yanti Yaacob
Pn. Lili Widarti Zainuddin

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Faculty of Applied Sciences,
Universiti Teknologi MARA,
Perak Branch Tapah Campus,
35400 Tapah Road,
Perak, Malaysia.

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

Rosliza Ali
Nunshaimah Salleh
Norsakina Zurina Zulkifli
Adibatul Husna Fadzil
Yanti Yaacob
Lili Widarti Zainuddin

Universiti Teknologi MARA
Perak Branch Tapah Campus
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ANTIBACTERIAL ACTIVITY OF *Piper betle* LEAVES EXTRACT AGAINST *Bacillus subtilis* AND *Escherichia coli*

Husna Zulkipli*, Nor Farhana Syamimi Mohd Syukri, Siti Nur Azmu'i Abdullah, Mohd Rabani Yaafar
Faculty of Applied Sciences, Universiti Teknologi MARA, Perak Branch Tapah Campus,
35400 Tapah Road, Perak, Malaysia.

*husna_zulkipli@uitm.edu.my

Abstract: This study investigates the antibacterial activity of *Piper betle*, a medicinal plant extensively used in traditional medicine across South and Southeast Asia. The research aims to evaluate the effectiveness of *P. betle* extracts against two bacterial strains: *Bacillus subtilis* and *Escherichia coli*. The study further explores the impact of different extraction times (12, 24 and 36 hours) on the antibacterial efficacy of the plant. Using the agar-well diffusion method, the antibacterial activity was assessed by measuring the inhibition zones around the bacterial cultures. The findings revealed that *P. betle* extracts exhibit significant antibacterial activity, with the highest inhibition zones observed at 24 hours of extraction. The study also employed Fourier-Transform Infrared Spectroscopy (FTIR) analysis to confirm the presence of bioactive compounds in the extracts such as flavonoids. These compounds are known to disrupt bacterial cell walls and inhibit protein synthesis, contributing to the plant's antimicrobial properties. The results suggest that *P. betle* holds potential as a natural alternative to synthetic antimicrobial agents, offering promising applications in healthcare, particularly in addressing the challenges of antibiotic resistance. Further research is recommended to optimize extraction methods and quantify the bioactive compounds for broader applications.

Keywords: *Piper betle, antibacterial activity, flavonoids, natural antimicrobial agent, traditional medicine*

INTRODUCTION

The increasing prevalence of antibiotic resistance and the adverse effects of synthetic chemicals in commercial sanitizers have sparked significant interest in the search for natural antimicrobial agents. Medicinal plants, which have long been a basis of traditional medicine, offer a promising alternative. One such plant is *P. betle*, commonly known as betel leaf or daun sireh, which is extensively cultivated in South and Southeast Asia for its therapeutic properties. *P. betle* has been traditionally used for its antimicrobial, anti-inflammatory, and antioxidant effects, making it a subject of interest in modern scientific research (Chaudhary et al., 2023). The antibacterial activity of *P. betle* is attributed to its rich content of bioactive compounds, particularly flavonoids. These compounds are known to disrupt bacterial cell walls, inhibit protein synthesis, and interfere with DNA gyrase, ultimately hindering bacterial growth and replication (Singh et al., 2023). Studies have demonstrated that *P. betle* exhibits broad-spectrum antibacterial activity against both gram-positive and gram-negative bacteria, highlighting its potential as a natural antimicrobial agent (Tran et al., 2023). Given the growing reliance on traditional medicine in many developing countries, where access to conventional healthcare may be limited, the exploration of *P. betle* as a natural antibacterial agent is particularly relevant. This study aims to evaluate the antibacterial properties of *P. betle*, focusing on the effects of different extraction times on bacterial inhibition. By identifying optimal extraction conditions, this research seeks to contribute to the development of natural alternatives to synthetic antimicrobial agents, addressing both the challenges of antibiotic resistance and the need for safer, more sustainable healthcare solutions (Sissi & Benzie, 2011).This investigation not only aims to validate the traditional use of *P. betle* but also to explore its potential applications in modern medicine, providing a bridge between traditional knowledge and contemporary scientific practices.

METHODOLOGY

Bacterial strain and culture media

The inoculum of bacteria *B. subtilis* and *E. coli* was prepared by picking well-isolated colonies from a pure culture plate. The inoculum was then spread evenly across the surface of the agar using the loop. The inoculated agar plates were then incubated at 37°C for 24 hours to allow the bacteria to grow.

Ethanolic extraction of *P. betle* leaves

The plant materials were washed, air-dried, and cut into smaller pieces. They were then oven-dried at 60°C for 5 days and ground into a coarse powder. The ground leaf was soaked in 70% ethanol. It was divided into three different extraction (soaked) times: 12 hours, 24 hours, and 36 hours. The leftover solvent was removed using a rotary evaporation to produce concentrated extract (Chaudhary et al., 2023).

Characterization of *P. betle* crude extracts

A Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer GX- FTIR) was used to identify functional group in the crude extracts. A small drop of the extracts was put on the sample holder. The infrared spectrum was then captured using the FTIR spectrometer across a range of wavelengths. The acquired spectrum was examined by comparing the absorption bands with recognized reference spectra to determine the functional groups in the extracts.

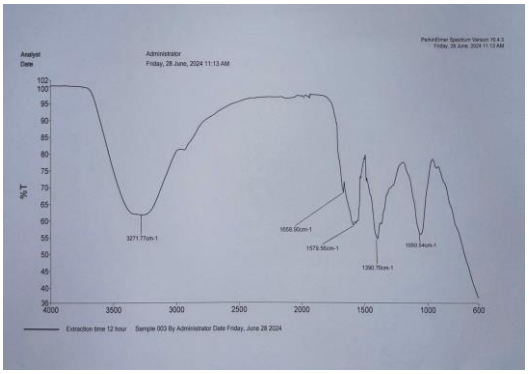
Antibacterial screening

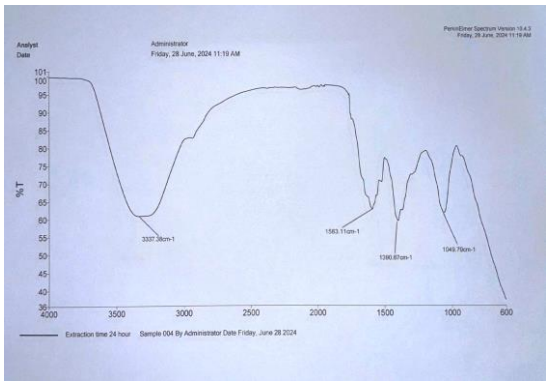
The method was conducted using agar well diffusion method. The inoculums of both bacteria were prepared. Agar was poured onto the petri dish containing the inoculum and thoroughly mixed using a swirling motion. After it solidified at room temperature, four wells of approximately 5 mm in diameter and 2.5 mm deep were made using the back tip of a sterile plastic pipette on the surface of the nutrient agar medium. Three of the four wells were pipetted with 50 µL of ethanolic leaf extracts, respectively, and the remaining well was used as a negative control using 50 µL of ethanol solution only. The steps were repeated for another 2 extracts of different extraction time (24 and 36 hours). All the plates were incubated at 37°C for 24 hours, and the size of the inhibition zone was measured for each well in centimeters (cm) using a ruler and converted into millimeters (mm) for the recording of results (Bhargav et al., 2016).

FINDINGS

Characterization of *P. betle* crude extracts

Figure 1 shows the FTIR analysis for A) 12 hours B) 24 hours and C) 36 hours extraction time. It was found that the functional group -OH band in Figure A, B and C respectively has the reading of 3271.77cm^{-1}, 3337.38 cm^{-1} and 3321.48 which were comparable to the finding of Nasution & Wulandari (2021). The FTIR analysis revealed the presence of hydroxyl (OH) groups in the *P. betle* extracts, which are indicative of phenolic compounds. These OH groups are known for their ability to interact with bacterial cell walls, leading to increased membrane permeability and subsequent bacterial cell death. This is consistent with the observed antibacterial activity, where significant inhibition zones were noted against both gram-positive and gram-negative bacteria, underscoring the role of phenolic compounds in the antimicrobial efficacy of *P. betle* (Jamil et al., 2021, Lubis et al., 2020).

A) 

B) 

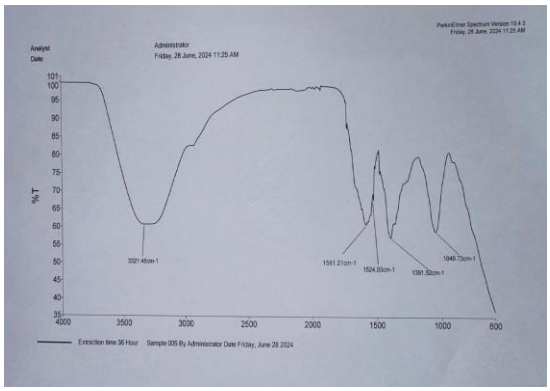
C) 

Figure 1. FTIR spectra of extraction at three different time points A) 12 hours B) 24 hours and C) 36 hours

Antibacterial activity

Figure 2 A) and B) shows the agar plates cultured with treated *B. subtilis* and *E. coli* respectively and treated with *P. betle* extracts of prepared different time points of extraction. Both agar plates show circular and clear inhibition zone of *P. betle* extracts against *B. subtilis* and *E. coli.* The inhibition zones were measured, with the highest zones observed at 24 hours of extraction. For *B. subtilis*, the inhibition zone was 2.67 cm, while for *E. coli*, it was 2.73 cm.

It was observed that the inhibition zone was the highest at 24 hours of extraction process and decreased at 36 hours (Figure C). This could be explained by Fick's second law of diffusion predicting that a final equilibrium between the solute concentration in the solid matrix (plant matrix) and in the bulk solution (solvent) might be reached after a certain time, leading to deceleration in the extraction yield (Silva et al., 2007). Moreover, prolonged extraction time increases the chance of decomposition and oxidation of phenolics due to their long exposure to unfavourable environmental factors such as temperature, light and oxygen (Naczk & Shahidi, 2004).

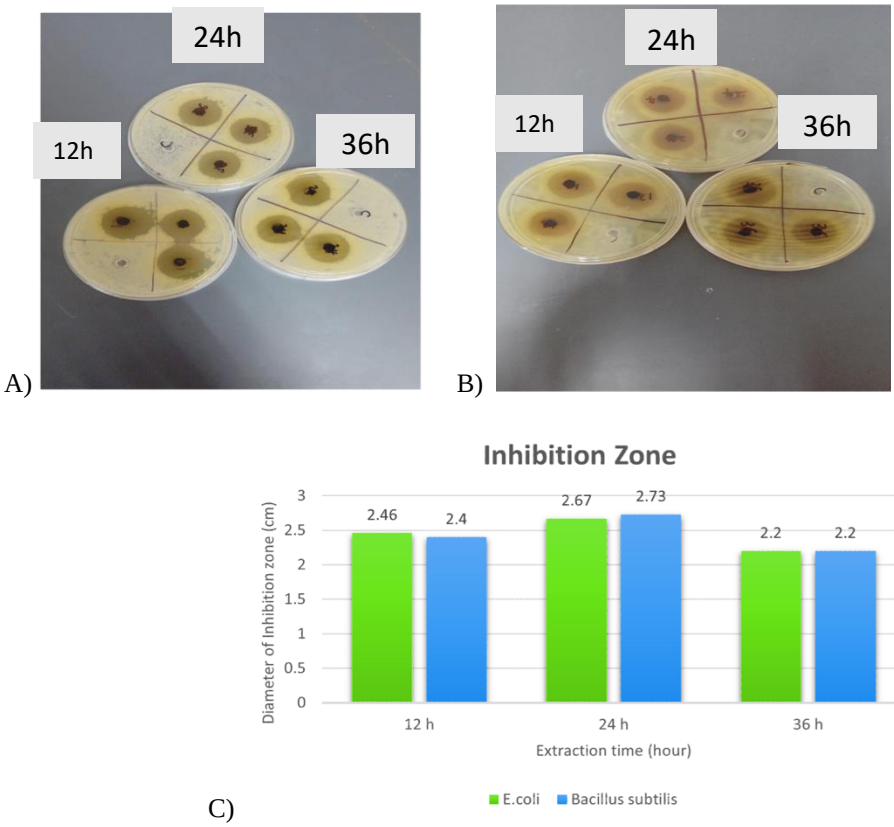


Figure 2. A) Inhibition zone of different time points of extraction of *P. betle* extracts against *B. subtilis.* B) Inhibition zone of different time points of extraction of *P. betle* extracts against *E. coli.* C) Comparison of inhibition zone of different extraction time of *P. betle* against *B. subtilis* and *E. coli.*

CONCLUSIONS

In conclusion, the study has provided valuable insights into the potential of time-based ethanolic extraction of *P. betle* as pathogenic inhibitory agents. The positive antimicrobial activity demonstrated against pathogenic bacteria *B. subtilis* and *E. coli*, highlights the promising nature of the extracts. This study supports the potential of *P. betle* as a natural antibacterial agent, particularly in the context of increasing antibiotic resistance. The findings align with previous studies, further validating the traditional use of *P. betle* for its medicinal properties. Further optimization of extraction methods could enhance the antibacterial efficacy of the plant extracts.

COMPLIANCE OF ETHICAL STANDARDS

Not applicable.

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Sekian, terima kasih.

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