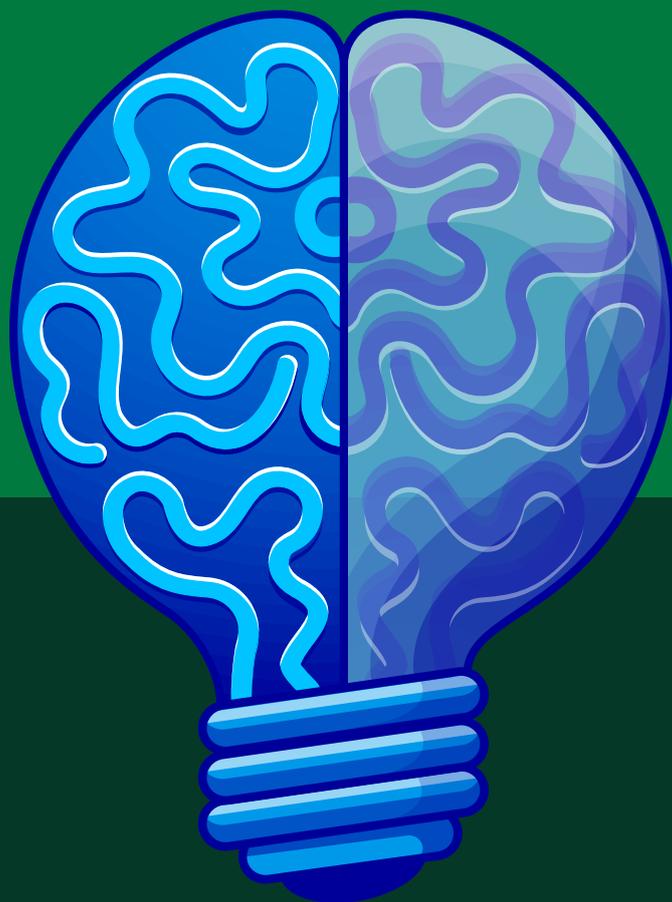


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UNIVERSITI TEKNOLOGI MARA
PERAK BRANCH

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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.

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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 cm^{-1} range at a resolution of 4 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 (H_2SO_4) was added. The mixture was examined for the development of a reddish-brown interface between the sulphuric acid (H_2SO_4) and chloroform (CH_2Cl) 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 (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 μL 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 (≤ 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.

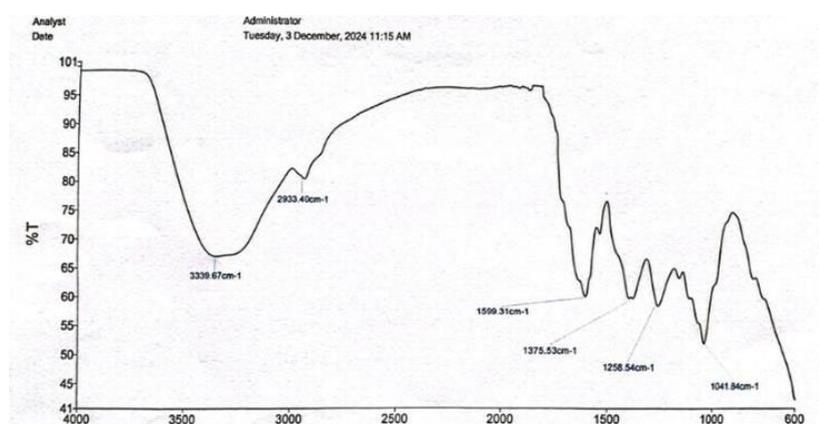


Figure 1 FTIR peaks from ethanolic mulberry leaf extract

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

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

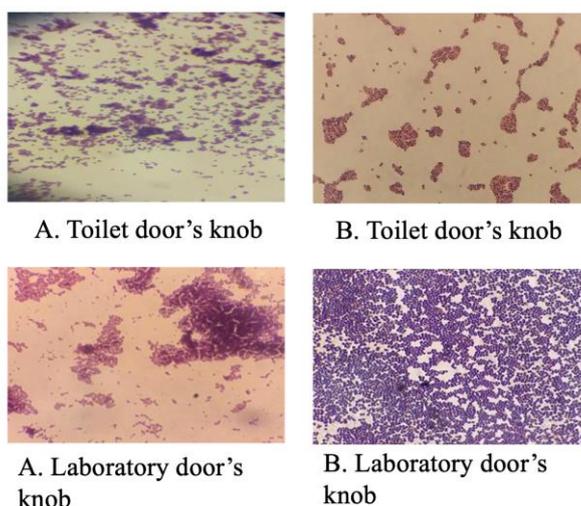


Figure 2 Gram-stained bacterial isolates collected from door handles and visualized under a light microscope at 100× magnification. (A) Bacterial smear from a toilet door's knob showing Gram-positive cocci in clusters. (B) Another view of the toilet door's knob, isolated with densely aggregated Gram-positive 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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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.

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