ORIGINAL ARTICLE

Antimicrobial Potential of *Garcinia atroviridis* Leaves Extract Against a Representative Panel of Bacterial Strains

Aini Nabihah Mohd Rosdi [1], Nurul Nabilah Mohd Rasdi [1], and Hartini Yusof [1]*

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

ABSTRACT

Introduction: Plants are getting a closer look for new medicines because existing drugs are becoming less effective against bacteria due to the misuse of antibiotics. This study aims to determine the antibacterial properties of *Garcinia atroviridis (G. atroviridis)* leaves extract against *Streptococcus mutans, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus mirabilis*. **Methods:** The antibacterial activity of the extract was assessed using the antibiotic susceptibility testing (AST) of the well-diffusion method to identify the zone of inhibition, the broth microdilution method to determine the minimum inhibitory concentration (MIC), and the streaking method to obtain the minimum bactericidal concentration (MBC). A qualitative test was used to examine the extract's phytochemical components. **Results:** The methanolic extract of *G. atroviridis* leaves showed significant activity against selected bacteria. The AST test showed the highest inhibition against *S. mutans* (37.67 mm) and the lowest inhibition against *P. aeruginosa* (17.00 mm). Based on the MIC result, gram-positive bacteria (*S. mutans and S. pyogenes*) inhibit at the lowest concentration of 1.95 mg/ml of leaves extract, while gram-negative (*P. aeruginosa and P. mirabilis*) inhibit at the highest concentration of 31.25 mg/ml. The MBC values of all tested organisms were similar to their corresponding MIC values. The phytochemical compounds discovered in the methanolic extract of *G. atroviridis* leaves include flavonoids, alkaloids, saponins, tannins, and terpenoids. **Conclusion:** The preliminary findings showed that *G. atroviridis* leaves could be an alternative antibacterial agent to combat bacterial infections.
Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP5): 172–180. doi:10.47836/mjmhs.21.s5.23

Keywords: *Garcinia atroviridis* leaves, antibiotic susceptibility testing (AST), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), phytochemical compounds.

Corresponding Author:
Hartini Yusof, MSc
Email: hartinieyusof@gmail.com
Tel: 03-32584431/0196635985
Fax: 03-32584599

INTRODUCTION

The emergence of antibiotic-resistant bacteria poses a significant threat to global health. Excessive and improper use of antibiotics has rendered many existing treatments ineffective (1). This has necessitated the discovery and development of novel antibiotics. However, traditional approaches often rely on modifying existing antibiotics, which can lead to microbes quickly developing resistance to the new drugs as well (2). Studies have shown widespread antibiotic resistance among bacteria, including *Streptococcus mutans* (3) and *Propionibacterium acnes* (4), highlighting the need for new antimicrobials. Plants have emerged as a promising source of new antibacterial agents.

Plants have been a rich source of natural medicines throughout history and hold immense potential for developing new antimicrobials. *Garcinia atroviridis*, also known as asam gelugor, is a Southeast Asian plant with a growing body of research exploring its potential health benefits. Studies suggest various pharmacological properties associated with different parts of the plant, including antifungal, antioxidant, and antidiabetic effects (5, 2).

Intriguingly, research suggests that *G. atroviridis* may also possess antibacterial properties. Extracts from its leaves, fruits, and stem bark have demonstrated activity against various bacterial and fungal strains (6, 2). The specific compounds responsible for this activity remain under investigation, but phenolic and flavonoid content are suggested to play a role (7). Notably, the antibacterial efficacy of these extracts can vary depending on the plant part used and the extraction method employed (5, 6).

This study focuses on the potential of *G. atroviridis*

leaves as a source of natural antibacterial agents. By investigating the antibacterial activity and identifying the phytochemical components responsible for methanolic extracts of *G. atroviridis* leaves, this research aims to contribute to the growing knowledge on this plant's potential as a novel weapon against antibiotic-resistant bacteria.

MATERIALS AND METHODS

Plant Collection

G. atroviridis leaves were collected from a site located at 3°51'4" North and 102°29'33" East in Felda Kesidang (Jengka 8), Bandar Pusat Jengka, Pahang, Malaysia. Only healthy leaves with consistent colour and appearance were selected. These leaves were then transferred to the laboratory at room temperature in a clean, large plastic bag.

Preparation of *G. atroviridis* Leaves Extract

Freshly collected *G. atroviridis* leaves were rinsed with tap water to remove adhering debris such as sand and dust. Following rinsing, the leaves were air-dried at room temperature for a set timeframe (e.g., 24 hours) to remove excess surface moisture. Subsequently, the leaves were oven-dried at 30°C for 48 hours (8). After drying, the leaves were mechanically ground into a fine powder using a suitable grinding apparatus (e.g., mortar and pestle, grinder). The resulting powder was stored in airtight containers at room temperature with proper labelling for identification.

Maceration and Extraction

One hundred grams of the *G. atroviridis* leaves powder was weighed accurately using an analytical balance (PW124, AE Adam, UK) and macerated with 1000 mL of absolute methanol (extraction solvent) in a 1000 mL Schott bottle. The mixture was maintained at room temperature for seven days with intermittent shaking on a platform shaker (Incubator 100/Unimax 1010 DT, Heidolph, German) to facilitate efficient extraction. Following the maceration period, the methanolic extract was sequentially filtered through a muslin cloth sieve followed by Whatman No. 1 filter paper to remove any residual particulates. The collected filtrate was then transferred to a clean flask for further processing.

The filtrate was concentrated using a rotary evaporator (N-100SW D, Eyela, USA) operating at a reduced pressure of 205 mbar and a controlled temperature of 40°C. This process removes the solvent and yields the crude extract. The concentrated extract was transferred to a pre-weighed Schott bottle and stored at 4°C for subsequent analysis. The weight of the empty bottle was subtracted from the final weight to determine the exact yield of the crude extract. The percentage yield of the extraction process was also calculated.

Preparation of Stock Solution

A stock solution of the *G. atroviridis* leaves methanolic extract was prepared by dissolving the crude extract in 0.25% (v/v) Dimethyl Sulphoxide (DMSO) to achieve a desired concentration of 1000 mg/mL.

Bacterial Culture Identification

The methanolic extract of *G. atroviridis* leaves was evaluated for its antibacterial activity against a panel of bacteria comprising *S. mutans* (ATCC 25175), *S. pyogenes* (ATCC 12384), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 10145), and *P. mirabilis* (ATCC 7002). Bacterial cultures were revived by streaking onto blood agar (BA) and nutrient agar (NA) media, followed by incubation at 37°C for 24 hours. For gram-positive bacteria (*S. pyogenes* and *S. mutans*), incubation occurred aerobically. gram-negative bacteria were incubated in a CO_2 incubator overnight. Prepared subcultures were stored at 4°C until use in subsequent experiments.

Antimicrobial Susceptibility Testing (AST) by Agar Well Diffusion Method

The antibacterial activity of the *G. atroviridis* leaves methanolic extract was evaluated using the agar well diffusion method. Briefly, bacterial cultures were revived in Mueller-Hinton Broth (MHB) and adjusted to a 0.5 McFarland standard turbidity. Mueller-Hinton Agar (MHA) plates were divided into three sections: positive control, negative control, and sample. Following sterilisation with a cotton swab, each section was inoculated with the respective test bacteria to create a confluent lawn. Sterile wells were made in the negative control and sample sections using a borer. The sample received 20 µL of the 1000 mg/mL methanolic extract, while the negative control received 20 µL of 0.25% DMSO solution. Selection of positive controls varied based on the test bacteria: chloramphenicol (30 µg) for *S. mutans*, gentamicin (10 µg) for *E. coli* and *P. mirabilis*, ciprofloxacin (5 µg) for *P. aeruginosa*, and penicillin (10 µg) for S. *pyogenes*. gram-negative bacteria were incubated at 37°C for 18-24 hours, while gram-positive bacteria (except *S. mutans*) were incubated in a CO2 incubator for 18-24 hours. *S. mutans* required 48 hours of incubation. Inhibition zone diameters around the wells were measured and recorded in millimetres. The experiment was performed in triplicate for each organism and data were presented as mean ± standard deviation.

Determination of Minimum Inhibitory Concentration (MIC)

The broth microdilution method determined the MIC of methanolic *G. atroviridis* leaves extract against the tested bacteria. A sterile 96-well U-bottom microtiter plate was employed for the assay. Bacterial suspensions were prepared by inoculating three to five colonies into 5 mL Mueller-Hinton broth (MHB) and incubating at 37°C until the log phase was reached. The turbidity of

the resulting suspension was adjusted to a 0.5 McFarland standard, equivalent to approximately 5 x 10^5 colony-forming units (CFU)/mL (9). A two-fold serial dilution of the leaves extract, ranging from 1000 to 1.95 mg/mL, was prepared in MHB. Fifty microliters of MHB were added to wells 2-10 of the microtiter plate, followed by 100 µL of the diluted extract in well 1. Subsequent two-fold serial dilutions were performed across wells 2-10. Fifty microliters of the bacterial suspension were then added to each well (1-10). Positive and negative controls were included, containing MHB and bacterial suspension and 0.25% DMSO in MHB, respectively. Plates were incubated at 37°C for 18-24 hours for gram-negative bacteria and at 37°C with 5% CO_2 for 18-24 hours for gram-positive bacteria, except *S. mutans*, which required 48 hours. The MIC was determined as the lowest extract concentration exhibiting no visible bacterial growth.

Determination of Minimum Bactericidal Concentration (MBC)

The MBC test was performed to confirm the MIC and to differentiate between bactericidal and bacteriostatic effects. Following the MIC determination, samples from microtiter plate wells with concentrations at or above the MIC and controls were evenly spread on Mueller-Hinton agar (MHA) plates using sterile cotton swabs. Plates were incubated overnight. The MBC was defined as the lowest extract concentration, resulting in no visible growth, indicating a ≥99.5% reduction in viable bacterial count compared to the initial inoculum (10).

Qualitative Phytochemical Analysis

Phytochemicals are plant-derived compounds with potential physiological effects on humans and serve as precursors for semi-synthetic pharmaceuticals (11). Standard colorimetric methods were employed to assess the phytochemical profile of the methanolic extract of *G. atroviridis* leaves. The extract was screened for flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids, and anthraquinones.

Flavonoids were detected through a positive alkaline reagent test, indicated by a yellow coloration. Alkaloids were precipitated using Mayer's and Wagner's reagents. Saponins formed stable foam upon shaking. Tannins reacted with ferric chloride to produce a greenish-black complex. Glycosides reduced Fehling's reagent to form a brick-red precipitate. Terpenoids were identified by a green colour change in the Liebermann-Burchard test. Finally, anthraquinones were detected through Borntrager's test and were characterised by a pink, red, or violet colouration.

Statistical analysis

The antimicrobial properties data of methanolic extract of *G. atroviridis* leaves was provided as the mean and standard deviation of three samples (n=3) as the test was done in triplicates for each bacteria. The data were analysed by using Statistical Package for the Social Sciences (SPSS) software version 28. An independent t-test was performed to compare the diameter of the inhibitory zone of leaves extract and standard antibiotics (positive control). It was determined that the results from the studies with p-value of less than 0.05 was statistically significant.

RESULTS

The Crude Methanolic *Garcinia atroviridis* Leaves Extract

Fig. 1 shows the results of the crude methanolic extract of *G. atroviridis* leaves by using rotary evaporator. The methanolic extract has characteristics of semi-liquid form, slightly sticky and viscous green-black colour. The extract obtained was 29.9 g from 100 g of *G. atroviridis* leaves powder. The extraction of *G. atroviridis* leaves resulted in a 29.9% extract yield. The extract yield was determined as w/w (%) = [Weight of yielded crude extract (g)/ Weight of *G. atroviridis* leaves powder (g)] x 100 %.

Fig. 1: The crude methanolic *G. atroviridis* leaves extract.

Antimicrobial Susceptibility Test (AST)

AST results presented in **Table I** and **Fig. 2** demonstrated that the methanolic *G. atroviridis* leaves extract exhibited inhibitory activity against all tested bacterial strains. Notably, *S. mutans* and *S. pyogenes* displayed significantly larger inhibition zone diameters of 37.67 ± 1.15 mm and 34.67 ± 1.53 mm, respectively. Conversely, *E. coli* and *P. mirabilis* exhibited smaller inhibition zones of 18.33 ± 0.57 mm and 18.33 ± 1.15 mm, respectively, followed by *P. aeruginosa* with an inhibition zone diameter of 17.00 ± 1.00 mm.

Table I: Mean of inhibition zone diameter of selected bacteria.

Organisms	Mean of inhibition zone diameter (mm) ± Standard deviation (SD)					
	Methanol extract (1000 mg/ml)	Positive control				Negative control
		C 30µg	P 10µg	G 10µg	CIP 5µg	
S.mutans	37.67±1.15	42.33± 2.08	-	-	-	*6.00± 0.00
S.pyogenes	34.67± 1.53	-	44.67± 1.53	-	-	*6.00± 0.00
E.coli	18.33± 0.57	-	-	24.00± 0.00	-	*6.00± 0.00
P.aerugi-nosa	17.00± 1.00	-	-	-	27.00± 0.00	*6.00± 0.00
P.mirabilis	18.33± 1.15	-	-	21.33± 0.57	-	*6.00± 0.00

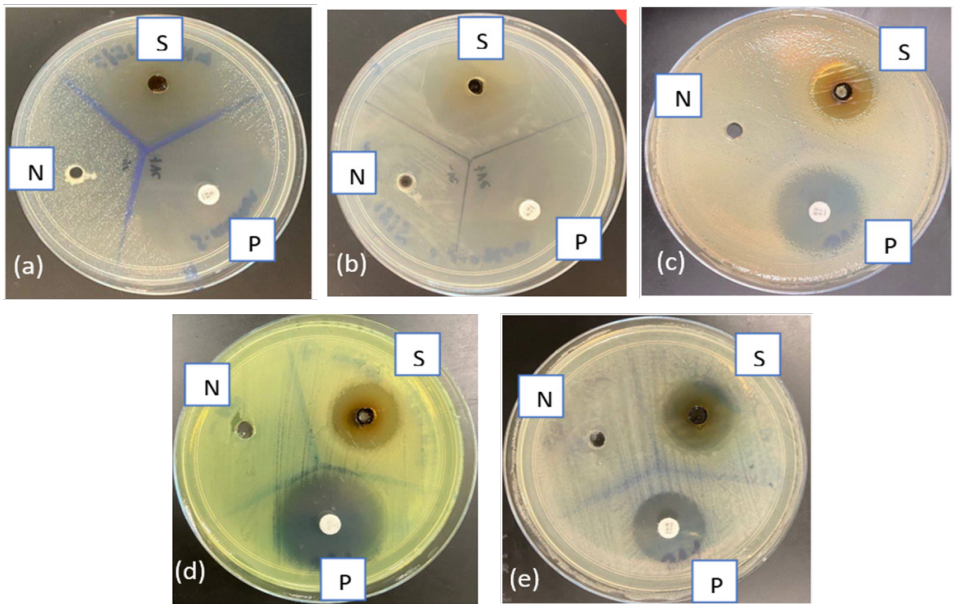

Fig. 2: AST plates of tested bacteria. (a) *S. mutans*, (b) *S. pyogenes*, (c) *E. coli*, (d) *P. aeruginosa*, (e) *P. mirabilis*, S: Sample (methanolic extract of *G. atroviridis* leaves (1000 mg/mL)), N: Negative control, P: Positive control.

Independent t-tests were conducted to assess the statistically significant differences in inhibition zone diameters between the methanolic *G. atroviridis* leaves extract and corresponding standard antibiotics for each bacterial strain. A significance level of $p < 0.05$ was adopted with a 95% confidence interval. As presented in **Table II**, all bacterial species exhibited p-values less than 0.05, indicating a statistically significant difference in inhibition zone diameters between the extract and the respective standard antibiotic.

Table II: Tabulation of independent t-test data comparing inhibition zone diameter between methanolic extract of *G. atroviridis* and standard antibiotic.

Tested organisms	Mean difference	95% Confidence Interval (CI)		t-test (df)	*P*-value*
		Lower	Upper		
S. mutans	-4.67	-8.48	-0.85	-3.39 (4)	0.027
S. pyogenes	-10.00	-13.46	-6.53	-8.018 (4)	0.001
E. coli	-5.67	-6.59	-4.74	-17.00 (4)	<.001
P. aeruginosa	-10.00	-11.60	-8.39	-17.32 (4)	<.001
P. mirabilis	-3.00	-5.06	-0.93	-4.02 (4)	0.016

P-value* <0.05, (df) degree of freedom

Determination of MIC and MBC Values

The methanolic *G. atroviridis* leaves extract demonstrated the lowest MIC value of 1.95 mg/mL against both *S. mutans* and *S. pyogenes* (**Table III**). In contrast, *P. aeruginosa* and *P. mirabilis* exhibited higher MIC values, requiring an extract concentration of 31.25 mg/mL for inhibition. *E. coli* showed an intermediate MIC of 15.63 mg/mL. Notably, the MBC values for all tested bacterial strains were equivalent to their respective MIC values.

Qualitative Phytochemical Analysis

Qualitative phytochemical analysis of the methanolic *G. atroviridis* leaves extract revealed the presence of flavonoids, tannins, alkaloids, saponins, and terpenoids, while glycosides and anthraquinones were absent (**Table IV**). The results, visually represented in **Fig. 3**, indicate the presence of these phytochemicals based on characteristic colour changes or precipitate formation.

DISCUSSION

This study investigated the phytochemical composition and antibacterial properties of *G. atroviridis* leaves against selected bacteria. Phytochemical extraction from plants involves multiple steps, including milling, grinding, homogenisation, and extraction (12). This study employed extraction to isolate phytochemicals from *G. atroviridis* leaves. Plant extraction is a process that involves recovering active compounds, or secondary metabolites, from plant material using a suitable solvent and standardized method. These compounds encompass a variety of substances, including alkaloids, flavonoids, terpenes, saponins, steroids, and glycosides (13). Several factors influence extraction yield, such as solvent polarity, pH, sample composition, extraction time, and temperature, with solvent and sample composition particularly critical (12).

The choice of solvent significantly impacts the antimicrobial activity of plant extracts, with polar solvents generally exhibiting higher antimicrobial efficacy than non-polar solvents (14). In this research, methanol was employed as the extraction solvent to obtain a crude extract from *G. atroviridis* leaves. The extraction yield from *G. atroviridis* leaves was substantial, reaching 29.9% w/w, resulting in 29.9 g of crude extract. Methanol, a commonly used extraction solvent due to its high polarity and low boiling point, was selected for this study. Its effectiveness in producing high extraction yields is well-documented (15). The obtained yield aligns with previous research demonstrating the superiority of methanol over ethanol and distilled water for extracting

Table III: MIC and MBC values of methanolic extract of *G. atroviridis* leaves against selected bacteria.

	Leaves extract concentration (mg/ml)				
	S. mutans	*S. pyogenes*	*E. coli*	*P. aerugi-nosa*	*P. mirabilis*
MIC	1.95	1.95	15.63	31.25	31.25
MBC	1.95	1.95	15.63	31.25	31.25

Table IV: Phytochemical screening of methanolic extract of *G. atroviridis* leaves.

Phytochemical test	Reaction
Flavonoid test	+
Tannins test	+
Alkaloids test	+
Saponins test	+
Terpenoids test	+
Glycosides test	-
Anthraquinones test	-

+: Positive/ present, -: Negative/ absent

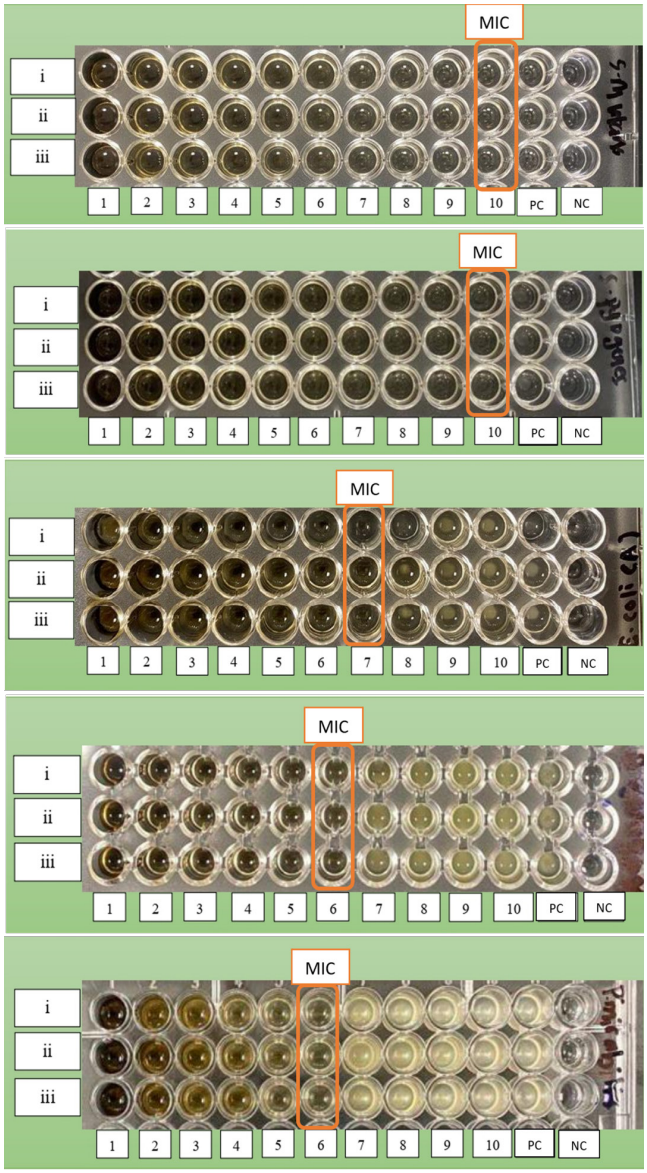

Fig. 3: The MIC (mg/ml) plates of methanolic extract of *G. atroviridis* leaves against (a) S.*mutans*, (b) S.*pyogenes*, (c) *E.coli*, (d) *P.aeruginosa* and (e) *P.mirabilis*. PC: positive control, NC: negative control, the test was done in triplicates (i, ii, iii).

compounds from *Polygonum minus* (15). Methanol is also recognized as an efficient solvent for extracting antibacterial compounds from plants. A comparison of methanol, n-hexane, and dichloromethane for extracting antimicrobial constituents from *Pelargonium graveolens* revealed methanol's superior performance due to its ability to extract higher concentrations of compounds like phenolics, flavonoids, tannins, and flavonols (16). The phytochemical analysis of the methanolic *G. atroviridis* leaves extract revealed the presence of compounds with potential medicinal and physiological activities. These compounds are crucial for developing novel antimicrobial agents, as they often exhibit unique mechanisms of action (6). Given the increasing prevalence of antibiotic resistance, the plant kingdom has emerged as a promising source of alternative antimicrobial compounds (11).

The phytochemical profile of the *G. atroviridis* leaves extract included flavonoids, alkaloids, saponins, tannins, and terpenoids, consistent with previous studies on other *G. atroviridis* plant parts such as fruits, rinds, and stem bark (17-19). Flavonoids, in particular, exhibit antibacterial properties by inhibiting DNA gyrase, an enzyme essential for DNA replication (20). This inhibition leads to DNA damage and cell death (21). Additionally, flavonoids can induce bacterial cell lysis by disrupting cell membrane integrity (20-23). Saponins and terpenoids also contribute to the antimicrobial activity of the extract. Saponins form complexes with cholesterol in the cell membrane, leading to cell lysis (24). Their amphiphilic nature facilitates the penetration of microbial membranes (25). Moreover, saponins can synergize with antibiotics, potentially addressing antibiotic resistance (26).

Alkaloids within the plant extract exhibit antimicrobial properties by disrupting bacterial cell wall integrity. By interfering with peptidoglycan synthesis, alkaloids prevent proper cell wall formation (18, 27). A functional cell wall is crucial for bacterial survival, protecting against osmotic lysis and external stressors. Additionally, alkaloids can intercalate with bacterial DNA, inhibiting mRNA transcription and further compromising cell viability (28). Tannins also contribute to antibacterial activity by binding to bacterial proteins, leading to protein coagulation or precipitation, thereby depriving bacteria of essential nutrients (18, 29). Tannins have also been reported to inhibit bacterial adhesion (30, 31). The bacteria included in this study are well-recognized pathogens associated with wound infections (30-33) and bloodstream infections (bacteremia) (34-38). These pathogens have developed resistance to multiple antibiotics, posing a significant global health challenge. For instance, *S. mutans* has demonstrated resistance to penicillin, tetracycline, and ceftriaxone (39), while *S. pyogenes* has exhibited resistance to clindamycin, ceftriaxone, and vancomycin (40). Among gram-negative bacteria, *E. coli* has developed resistance

to ampicillin, cefepime, and cefoxitin (41), while *P. mirabilis* has shown resistance to tetracycline, ampicillin, and chloramphenicol (42). *P. aeruginosa* is known to be resistant to gentamicin, amikacin, and ciprofloxacin (43). Susceptibility testing demonstrated that the methanolic *G. atroviridis* leaves extract was effective against all five bacterial strains. Notably, the extract exhibited more potent antibacterial activity against gram-positive bacteria (*S. mutans and S. pyogenes*) than gram-negative bacteria (*E. coli, P. aeruginosa, and P. mirabilis*). These findings align with previous research reporting higher inhibitory activity of methanolic Indigofera oblongifolia leaves extract against gram-positive bacteria, as indicated by larger inhibition zone diameters than gram-negative bacteria (44). Similarly, ethanolic extracts of *Syzygium aromaticum* have shown potent antibacterial effects against gram-positive pathogens like *Bacillus cereus* and *Staphylococcus aureus*, responsible for food poisoning, with reduced efficacy against gram-negative bacteria such as *E. coli, P. aeruginosa*, and *S. typhi.*

Consistent with previous research demonstrating the potent antibacterial activity of *S. aromaticum* leaves extract against gram-positive bacteria (45). Thongkham et al. (2021) reported higher efficacy of ethanolic *G. atroviridis* fruit extract against gram-positive compared to gram-negative bacteria. While the methanolic *G. atroviridis* leaves extract exhibited activity against gram-positive and gram-negative bacteria in the present study, its mechanism of action likely involves cell membrane disruption. Gram-positive bacteria, lacking an outer membrane, are more susceptible to this mechanism compared to gram-negative bacteria, which possess a protective outer lipid barrier (6, 46).

The MIC assays demonstrated that the gram-positive bacteria *S. mutans* and *S. pyogenes* exhibited lower MIC values (1.95 mg/ml) compared to the gram-negative bacteria *P. aeruginosa* and *P. mirabilis* (31.25 mg/ml), with *E. coli* displaying an intermediate MIC (15.63 mg/ml). The correlation between MIC and MBC values indicated the bactericidal nature of the extract against both gram-positive and gram-negative organisms. Statistical analysis using an independent t-test revealed significantly larger inhibition zones for the methanolic extract compared to the standard antibiotic ($p < 0.05$) for all tested bacteria. However, these zones were slightly smaller than those observed for the positive control, aligning with previous studies using methanolic extracts of *G. atroviridis* fruits (1) and stem bark (6).

The slightly smaller inhibition zones observed for the methanolic extract of *G. atroviridis* leaves compared to the positive control suggest moderate antibacterial activity relative to commercial antibiotics. Solvent selection significantly influences the extraction efficiency of antibacterial compounds from plants. While methanol was used in this study, ethanol has been reported to effectively extract phytochemicals such as steroids, flavonoids, and terpenoids from *G. atroviridis* (2). Other factors, including pre-incubation diffusion, can impact extraction efficiency. For optimal diffusion of extract compounds into the agar during antimicrobial susceptibility testing (AST), refrigerating the plates after the application of the extract is recommended (47). Additionally, prolonged maceration times can negatively affect extract quality, leading to compound degradation (48, 49). To prevent this, it is crucial to remove the methanol solvent from the filtrate after seven days of maceration and avoid prolonged storage before concentration. Enhancing the maceration process through frequent stirring or agitation can improve solvent-plant contact, thereby increasing the release of bioactive compounds (50).

CONCLUSION

The current study demonstrated the antibacterial potential of a methanolic extract of *G. atroviridis* leaves, suggesting its utility in combating antibiotic resistance. While the extract exhibited antibacterial activity against both gram-positive and gram-negative bacteria, its efficacy was notably higher against gram-positive strains, as evidenced by larger inhibition zones. MIC values further corroborated these findings, with gram-positive bacteria demonstrating greater susceptibility to the extract. The bactericidal nature of the *G. atroviridis* leaves extract, as indicated by the correlation between MIC and MBC values, suggests its potential as a promising candidate for alternative antibacterial therapy. However, further research is needed to evaluate its effectiveness. The presence of phytochemicals such as flavonoids, alkaloids, saponins, tannins, and terpenoids within the extract may contribute to its observed bioactivity.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

ACKNOWLEDGEMENTS

The authors would like to acknowledge Universiti Teknologi MARA (UiTM) for supporting this research.

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