

Research Article



Phytochemical profiling and anti-*Staphylococcus epidermidis* activity of mango leaf extract using gas chromatography-mass spectrometry

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Article Information	ABSTRACT
Article History: Submitted: 2025-02-06 Revision: 2025-06-01 Accepted: 2025-07-18 Published: 2025-07-18	<i>Staphylococcus epidermidis</i> is increasingly recognized for its resistance to certain antibiotics, posing a significant threat due to its potential to cause infections. Plant extracts have emerged as promising alternatives owing to their diverse phytochemical composition. This study aimed to evaluate the antibacterial efficacy of the ethanolic extract of <i>Arumanis</i> mango leaves (<i>Mangifera indica</i> L. var. <i>arumanis</i>) against <i>S. epidermidis</i> and to characterize its metabolite profile using Gas Chromatography-Mass Spectrometry (GC-MS). Employing the disc diffusion method, the extract demonstrated inhibitory activity against <i>S. epidermidis</i> at a concentration of 2% (2 mg/ml). Subsequent GC-MS analysis identified <i>phenols</i> , <i>flavonoids</i> , and <i>alcohols</i> as the predominant bioactive compounds, consistent with their known antibacterial properties. These findings suggest that <i>Arumanis</i> mango leaf extract holds potential as a natural antibacterial agent against <i>S. epidermidis</i> and warrants further investigation for incorporation into natural antibacterial formulations.
Keywords: Antibacterial; mango; MIC; <i>Phenols</i> ; <i>Staphylococcus epidermidis</i>	
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INTRODUCTION

Staphylococcus epidermidis, an opportunistic human pathogen, has emerged as the most prevalent cause of nosocomial infections and lead serious complications (Vuong & Otto., 2002; Lee & Anjum., 2023). In addition, *S. epidermidis* frequently infects the skin, causing acne inflammation (Meilina & Hasanah, 2018; Hafsari et al., 2015). *S. epidermidis* has been recorded to resist antibiotics such as methicillin and penicillin (Lee et al., 2018). Antibacterials derived from plant extracts are a solution to antibiotic resistance in gram-positive and gram-negative bacteria (Frieri et al., 2017). Also, according to

WHO, finding plant antibiotic alternatives is an emergency. Researchers are exploring the potential of natural antibacterials derived from plant extracts as a substitute for antibiotics (Ningsih et al., 2023; Yodha et al., 2023). These natural antibacterials can impede the growth of harmful bacterial strains (Siregar et al., 2012).

Arumanis mango (*Mangifera indica* L. var. *arumanis*) (hereafter *Arumanis*) are a notable plant species with medicinal potential. A wide range of mango plant parts have been utilized in customary medicinal practices, including the leaves, bark, and fruit, with the belief that the plant exhibits characteristics that contribute to the treatment of diverse health concerns, such as diarrhea, dysentery, fever, respiratory issues, and stomach problems (Tollenaere et al., 2022; Shah et al. 2010). Hidayati et al., (2022) proved that the arumanis mango leaves have antiinflammatory effect (EEML) in vitro and in vivo, respectively. Moreover, mango leaves also have been used to help skin issues causing by bacteria (Ningsih et al., 2021; Hepziba et al., 2023). The antibacterial activity of mango leaf ethanol extract (*Mangifera indica* L.) has been demonstrated through experimental testing on various bacterial strains, including gram-positive bacteria such as *Staphylococcus aureus*; *C. acne* and gram-negative bacteria such as *Shigella flexneri*, *Escherichia coli* (Kumalasari et al., 2024; Tollenaere et al., 2022; Ningsih et al., 2020). In addition, the antibacterial efficacy of the extract has been observed against various fungi (Mehmood, H., Mehmood, J., & Zulfiqar, N., 2024). Bioactivity as antibacterials due to the presence of metabolites such as *flavonoids*, *alkaloids*, *saponins*, *quinones* or *anthraquinones*, *steroids*, and *tannins* (Tollenaere et al., 2022; Diso et al., 2017; Samrot et al., 2016; Zakiah et al., 2023). The presence of phytochemicals within *Arumanis* mango leaf extracts accounts for their antibacterial, anti-inflammatory, and antioxidant effects. Consequently, phytochemical profiling of mango plants is imperative to ascertain the comprehensive metabolome of the mango plant. Leaves are often more antibacterial than other plant components (Mehmood, H., Mehmood, J., & Zulfiqar, N., 2024).

Gas Chromatography-Mass Spectrometry (GC-MS) is a widely used technique in metabolite profiling to identify and characterize bioactive compounds of plants (Putri et al. 2013). It is particularly effective in detecting volatile and semi-volatile secondary metabolites, such as *terpenoids*, *phenolics*, and *alkaloids*, which have antibacterial properties (Shah et al., 2023). The advantage of GC-MS lies in its ability to separate complex mixtures, provide specific mass spectra for each compound, and enable accurate quantification. This enables comprehensive analysis of the metabolome components that contribute to antibacterial mechanisms (Swamy et al., 2017; Chirumamilla, P., Dharavath, S. B., & Taduri, S., 2022). The objective of this study is to examine the antibacterial activity of mango leaves (*Mangifera indica*) against the growth of *Staphylococcus epidermidis*, a genus of *Staphylococcus* that has not been previously examined. GC-MS was employed to conduct metabolite profiling. Further research aimed to explore the potential of *Arumanis* mango leaf extract as an antibacterial agent and its efficacy in treating skin conditions.

RESEARCH METHODS

This laboratory-based experimental study investigates the interactions between *Arumanis* leaf tissue and a specific bacterial strain of *S. epidermidis* under controlled conditions. A total of 3 kg of mature and healthy mango leaves were selected for optimum yield of bioactive compounds. The collected leaves were meticulously sorted, washed, and air-dried for five days at room temperature in a shaded area. Subsequently, powdered leaves (simplicia) were obtained by blending the dried leaves. Sequential five day-maceration using ethanol (70%) was performed with regular stirring twice daily (Utami & Putri, 2020). Separating the resulting mixture into residue and filtrate, used fine filter paper, yielding the macerate.

Finally, the concentrated extract was obtained through rotary evaporation (RV 10 Basic V) with a water bath set at 40°C for one hour. For metabolite profiling, 1 µL of *ethanol* extract of *Arumanis* mango leaves was injected into the GC-MS machine (Shimadzu, QP2010 Plus). The GC analyses utilized a split/splitless injector (ratio 1:30) and a flame ionization detector, both operating at 290°C. High purity helium served as the carrier gas at a flow rate of 1 ml/min. The capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The column temperature was initiated at 60°C for three minutes, followed by a programmed increase to 220°C at a rate of 5°C/min and maintained at 220°C for five minutes. All data resulted aligned with Wiley 7 Library and the metabolite data are classified based on literature studies such as Pubchem database.

Furthermore, for bacterial preparation and culture, comprehensive sterilization of all research equipment and materials were conducted utilizing an autoclave set at standard conditions (1 atm, 121°C, for 15 minutes). *S. epidermidis*, Surabaya Health Laboratory Centre collections, were cultured on slanted Nutrient Agar (NA) medium. For rejuvenation purposes, a medium of 0.4 instance NA was dissolved in 15 mL of distilled water and heated. The bacteria were inoculated onto solidified medium using streaking methods and incubated at 37°C for 24 hours. Validation of bacteria was conducted using gram staining methods and *S. epidermidis* displayed a purple colour, as gram-positive bacteria, under microscope observation. For the minimum inhibition test, rejuvenated bacterial colonies were suspended in 10 mL of 0.9% NaCl, homogenized using a vortex mixer (KMC-1300V, Vision Scientific®), and standardized to McFarland 0.5 (108 colonies/mL). The ability of plant extract efficacy in inhibiting bacteria was performed through well diffusion method. Five series of ethanol extract of *Arumanis* mango leaves (P1=1 mg/ml (1%), P2=2 mg/ml (2%), P3=3 mg/ml (3%), P4=4 mg/ml (4%), and P5=5 mg/ml (5%)) were prepared, with 0,1 mg/ml *chloramphenicol* serving as the positive control and aquadest as the negative control (each treatment was replicated four times). Serial concentrations were established by diluting the stock solution following a dilution formula. The antibacterial test used the well-diffusion method, where 50 µL of each extract variation was placed into designated wells and subsequently incubated at 37°C for 24 hours. The Concentration Series used was determined by a preliminary test. Observations on 24-hour incubated bacteria exhibited a clear zone which indicates the sensitivity of the bacteria to the antibacterial compound. The clear zone expressed the area of inhibition. The area of inhibition was measured both vertically and horizontally using a calliper (mm). The inhibition data were utilized for extract classification effectiveness (Table 1) (Surjowardojo and Susilorini, 2025). The One-Way ANOVA and Duncan's further test analyzed data results using SPSS version 26.

Table 1. Category of Inhibition Zone

Clear Zone Diameter	Category
≥ 21 mm	Extremely strong
10-20 mm	Strong
6-10	Medium
< 5	Weak

FINDING AND DISCUSSION

The GC-MS instrumentation revealed the presence of 31 metabolites, yet there are only 10 volatile organic compounds that meet a similarity index (SI) higher than 89% (Table 2). Similarity index less than 89% was not considered. The similarity index influences the estimation of compound identification based on the similarity of fragmentation patterns found in the library. A high SI value near 100% indicates that the identified compound closely resembles the standard compound.

Table 2. Phytochemicals of *Arumanis* Mango Leaves Extract

No	Compound Name	Retention time	Total (%)	Compound Group	SI (%)
1	2-Furanmethanol (CAS) Furfuryl alcohol	3.986	1.24	Alcohol	95
2	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	25.076	7.21	Flavonoids	93
3	Benzoic acid (CAS) Retardex	27.718	0.35	Phenols	92
4	2-Furancarboxaldehyde, 5-(hydroxymethyl)- (CAS) HMF	29.387	6.76	Alcohol	91
5	1,2-Benzenediol (CAS) Pyrocatechol	30.417	10.70	Phenols	95
6	1,4-Benzenediol (CAS) Hydroquinone	33.427	4.43	Phenols	93
7	1,2,3-Benzenetriol (CAS) 1,2,3-Trihydroxybenzene	37.403	2.93	Phenols	90
8	1,2,3-Benzenetriol (CAS) 1,2,3-Trihydroxybenzene	37.598	0.26	Phenols	92
9	1,2,3-Benzenetriol (CAS) 1,2,3-Trihydroxybenzene	37.941	6.18	Phenols	94
10	1,6-Anhydro-Beta-D-Glucopyranose (Levoglucosan)	39.754	1.38	Fatty Acids	95

Based on GC-MS results, the *Arumanis* mango leaves extract both contains primary and secondary metabolites that probably contribute to antibacterial activity. Displayed primary metabolites consist of alcohol groups and fatty acid. The group of secondary metabolites detected were 7 compounds classified into *phenols* and *flavonoids*.

The ethanol extract of *Arumanis* mango leaves has been shown to stop bacterial growth, as seen in the clear zone around the wells (Figure 1). The diameter of the inhibition zone at each concentration of *Arumanis* mango leaf *ethanol* extract had different effects. The data showed that the higher the concentration, the greater the inhibition zone. Table 3 shows the size of clear zone at each of treatments.

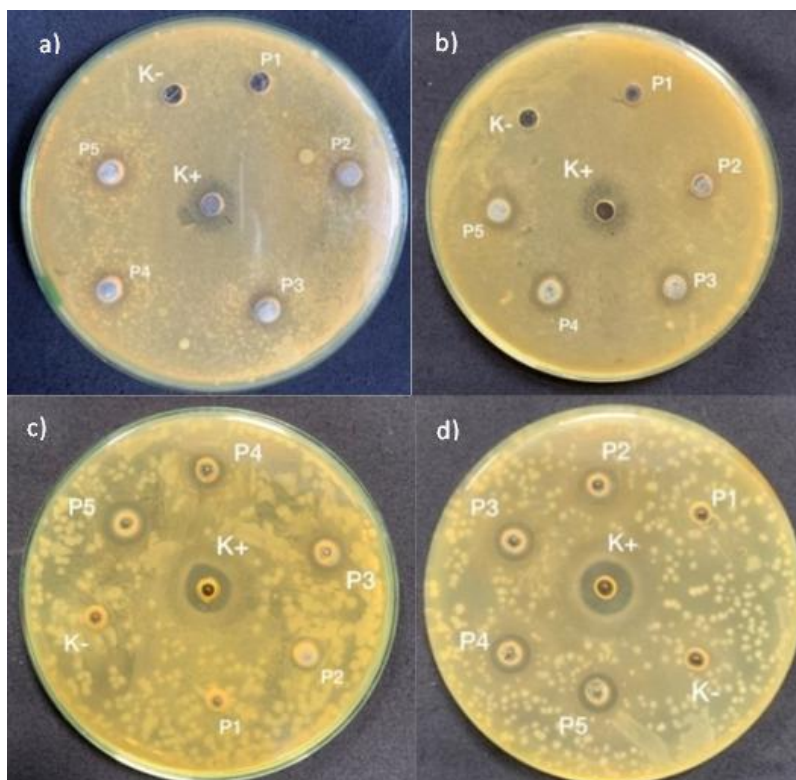


Figure 1. Antibacterial Test of Mango Leaf Extract to *S. epidermidis*, a-d Represents Repetition (Serial Dose: P1: 1%; P2: 2%; P3: 3%; P4: 4%; P5: 5%); K+: Chloramphenicol 0,1%; K-: Distilled Water

Mango leaf extract has antibacterial activity, as seen in its inhibition of *S. epidermidis* (Figure 1). This is more pronounced than distilled water, which exhibited no inhibitory effect. SPSS software revealed substantial variation in the inhibitory effect among the treatments (Pvalue < 0,05) (Table 3).

Table 3. Antibacterial Activity (Inhibition zone, mm) of Ethanol Extract of Mango *Arumanis* Leaf

Treatment	Mean Zone of Inhibition (mm) $\pm$ SD	Inhibition Response
K- (Distilled water)	0,00 $\pm$ 0,000 ^a	No inhibition
P ₁ (1% extract)	0,00 $\pm$ 0,000 ^a	No inhibition
P ₂ (2% extract)	3,05 $\pm$ 0,660 ^b	Weak
P ₃ (3% extract)	3,55 $\pm$ 0,500 ^b	Weak
P ₄ (4% extract)	3,95 $\pm$ 0,472 ^b	Weak
P ₅ (5% extract)	4,83 $\pm$ 0,88 ^{bc}	Weak
K+ (Chloramphenicol 0,1%)	7,73 $\pm$ 1,11 ^d	Medium

The lowest effective concentration of mango leaf extract was 2%. Below 2%, no clear zone was observed, indicating no inhibition of *Staphylococcus epidermidis* bacteria. The inhibitory power increased with increasing extract concentration. The inhibitory effect of mango leaf *ethanol* extract on various bacterial strains has been documented (Kumalasari et al., 2024; Tollenaere et al., 2022; Ningsih et al., 2021; Diso et al., 2017). Mango leaf extract, when combined with GC-MS analysis, contains *alcohol* and *hydroquinone* compounds. However, the *ethanol* extraction method has been found to yield *phenolic* groups and *xanthonones* (Mehmood, H., Mehmood, J., & Zulfiqar, N., 2024).

By the findings of the research, this study employed qualitative analysis to assess the composition of the *ethanol* extract of mango leaves. The analysis yielded a total of 10 valid compounds, which were identified using GC-MS instrumentation (Table 2). The identified compounds were found to be *phenolic*, *flavonoid*, *alcohol*, and *fatty acid* derivatives. Simple *phenols* and *phenolic acids* exhibit significant structural variability, ranging from unsubstituted to multiply substituted forms. *Polyphenols* represent a substantial and varied group of compounds, characterized by antioxidant properties that contribute to their antimicrobial effects (Othman L, Sleiman A and Abdel-Massih RM., 2019). The 3 abundant *phenol* compounds found in the ethanol extract of mango leaves are 1,2-Benzenediol (CAS) *Pyrocatechol*, 1,4-Benzenediol (*Hydroquinone*), and 1,2,3-Benzenetriol (CAS) *1,2,3-Trihydroxybenzene*. *Catechol* and its derivatives exhibit higher viral inhibition effects than other *phenolic* derivatives. Moreover, the leaves, which are traditionally known to be useful for infections, burns, and scalds, are linked to antioxidant, antimicrobial, antiviral, and antibacterial activities since they have a high polyphenol content (Mehmooda, Mehmoodb, Zulfiqara., 2024). The next metabolite, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one was a *flavonoid*. Data confirmed with other references that the methanol extract of arumanis mango leaves shows the presence of *flavonoids* (Ningsih et al., 2023). *Flavonoids* inhibit bacterial growth by damaging cell walls, inactivating enzymes, and damaging cell membranes (Nugraha, A. C., Prasetya, A. T., & Mursiti, S., 2017); (Ines Thabti et al., 2014). Further abundance by the presence of alcohol compounds namely 2-Furancarboxaldehyde, 5-(hydroxymethyl)-(CAS) *HMF*. *Alcohol* is any organic compound with a *hydroxyl* group (-OH) attached to a carbon atom, which is attached to another hydrogen atom and a carbon atom (Asngad & Nopitasari, 2020; Admojo, 2020). The activity of *alcohol* as an antibacterial comes from the content of *triclosan*, which is thought to affect the microbial cell wall so that the integrity of the bacterial cell wall is disrupted and causes the cell to lyse (Musetti et al., 2014). The possible inhibition mechanism of *ethanol* extract from mango leaf against *S. epidermidis* regarding the dominant compound contained can be illustrated in Figure 2.

Another salient factor in the mechanism of inhibition of mango leaf extract against *S. epidermidis* bacteria is its nature as a Gram-positive bacterium. Gram-positive bacteria possess a more simplified cell wall structure, characterized by an abundance of *peptidoglycan* (the primary component susceptible to antibacterial compounds) and a paucity of *lipids* (approximately 1–4%). along with the presence of *teichoic acid* (Diso et al., 2017; Lee et al., 2018).

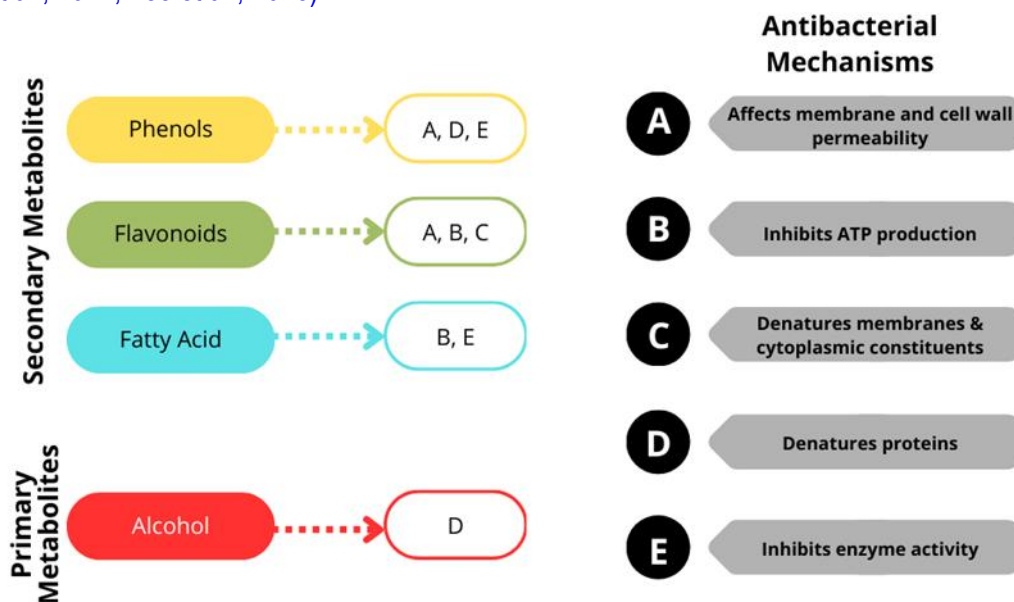


Figure 2. Possible Antibacterial Mechanisms (Represented As A-E) of Metabolites from *Ethanol* Extracts of *Arumanis* Mango Leaves

Moreover, the maceration method, employing 70% *ethanol* as a polar solvent, plays a pivotal role. It has been demonstrated that 70% ethanol is more effective at extracting *phenolic* compounds and other secondary metabolites that act as antioxidants compared to other solvents, such as *methanol*, due to their similar polar properties (Olusola et al., 2019). The extent of secondary metabolite extraction has been demonstrated to directly correlate with pharmacological activity (Nugraha, A. C., Prasetya, A. T., & Mursiti, S., 2017).

CONCLUSION

The *ethanol* extract of *Arumanis* mango leaves showed antibacterial activity against *S. epidermidis* with an inhibitory concentration as low as 2% (2 mg/ml). Phytochemical analysis indicates this effect is due to *phenol*, *flavonoid*, and *alcohol* compounds. While this finding suggests the extract's minimum inhibitory concentration is higher than conventional antibiotics, it provides fundamental insights into its metabolite composition. However, the small inhibition zones and higher effective concentration compared to conventional antibiotics highlight limitations. Therefore, *Arumanis* mango leaf extract holds potential as a natural antibacterial alternative, but further research is essential to understand its therapeutic value and potential applications.

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