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Antioxidant and Antibacterial Activities of Methanol and N-Hexane Extracts from *Mangifera indica* L. var. madu Leaves: The Role of Solvent Polarity

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Abstract

Leaves of *Mangifera indica* L. var. madu (locally known as mangga madu) are known to contain various secondary metabolites that have the potential as natural antioxidants and antibacterial agents. This study aimed to screen secondary metabolites, evaluate antioxidant activity, and determine the antibacterial activity of methanol and n-hexane extracts of mangga madu leaves against *Staphylococcus aureus* and *Escherichia coli*. The extracts were obtained by maceration and subjected to phytochemical screening, DPPH radical scavenging assay (IC_{50}), and disc diffusion antibacterial test at concentrations of 6.25%, 12.5%, 25%, and 50%. Phytochemical screening showed that the methanol extract contained alkaloids, flavonoids, tannins, and saponins, while the n-hexane extract contained steroids. The methanol extract exhibited very strong antioxidant activity with an IC_{50} of 33.48 μ g/mL, whereas the n-hexane extract showed very weak activity with an IC_{50} of 245.90 μ g/mL. Antibacterial testing revealed that the methanol extract produced inhibition zones up to 15.50 mm against *S. aureus*, while the n-hexane extract showed no inhibition at all concentrations. These findings indicate that the methanol extract of madu mango leaves has promising potential as a natural antioxidant and antibacterial agent, particularly against Gram-positive bacteria.

1. INTRODUCTION

Air pollution in urban areas has become a major environmental issue in Indonesia, particularly in cities with high population density. Exposure to airborne pollutants triggers the formation of reactive oxygen

species (ROS) or free radicals. These unstable molecules can damage essential cellular components, including proteins, lipids, carbohydrates, and DNA [1]. This oxidative damage contributes to the development of various degenerative diseases, including

cancer, cardiovascular disorders, and premature aging [2]. Natural antioxidants derived from plants offer a safer alternative to synthetic antioxidants, some of which have been associated with carcinogenicity [3].

Simultaneously, antibiotic resistance has emerged as a critical global health threat. This condition occurs when bacteria develop mechanisms to survive exposure to antibiotics that would normally kill them or inhibit their growth [4]. Both Gram-positive bacteria such as *Staphylococcus aureus* and Gram-negative bacteria such as *Escherichia coli* have shown resistance to commonly used antibiotics, including methicillin, ampicillin, and amoxicillin [5]. The consequences of antibiotic resistance include increased mortality rates, prolonged hospital stays, higher healthcare costs, and limited therapeutic options for infectious diseases [6]. These challenges have intensified the search for novel antibacterial agents from natural sources.

Mangifera indica L., commonly known as mango, is one of Indonesia's most widely cultivated fruit trees, with numerous varieties distributed across the archipelago. Beyond its popular fruit, various parts of the mango have been used in traditional medicine for wound healing and antidiabetic treatments. Several studies have confirmed that mango leaves contain diverse secondary metabolites including flavonoids, phenolics, tannins, saponins, alkaloids, and triterpenoids, which contribute to their pharmacological activities [7]. The specific variety and plant part significantly influence the composition and biological activities of these compounds [8].

Previous research has demonstrated the potential of various mango varieties as sources of antioxidants and antibacterial agents. Pridaningtias et al. [9] reported that methanol extract of *M. indica* var. podang exhibited very strong antibacterial activity against *E. coli*, while Hanifa et al. [10] showed that *M. indica* var. harum manis effectively inhibited *S. aureus* growth. Nugroho [11]

found that *M. indica* var. harum manis possessed strong antioxidant activity with an IC_{50} of 89.73 $\mu\text{g/mL}$. These findings highlight the importance of exploring different mango varieties to discover novel bioactivities.

Mangifera indica L. var. madu, known in Indonesia as mangga madu, is a unique variety prized for its sweet fruit. Fachriyah et al. [12] reported that the ethanol extract of *M. indica* L. var. madu leaves exhibited strong antioxidant activity (IC_{50} = 79.73 ppm). However, several research gaps remain. Although the antioxidant activity of this variety has been documented, its antibacterial activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli* has not been determined. Moreover, no previous study has compared polar and non-polar extracts of this variety, so it remains unclear whether solvent polarity alters the secondary metabolite profile and the resulting antioxidant and antibacterial activities. In addition, the relationship between extract concentration and antibacterial efficacy against Gram-positive and Gram-negative bacteria has not been characterized, leaving the effective concentration range undefined. This study was therefore designed to address these unresolved questions.

The selection of an appropriate extraction solvent is critical because it governs not only the solubility of target metabolites but also extraction efficiency, selectivity, and the chemical integrity of the resulting extract. The "like dissolves like" principle provides only a starting point: polar solvents such as methanol generally extract polar to semi-polar compounds (e.g., alkaloids, flavonoids, tannins, saponins), whereas non-polar solvents such as n-hexane favor non-polar compounds (e.g., steroids, terpenoids) [13-14]. However, solvent polarity also influences the ability of the solvent to penetrate the plant matrix, the release and diffusion of intracellular compounds, and the co-extraction of non-target matrix components such as pigments, lipids, waxes, and sugars, which can interfere

with antioxidant and antibacterial assays [15]. Furthermore, solvent properties (e.g., dielectric constant, viscosity, and volatility) and extraction conditions can affect the stability of labile metabolites; flavonoids, tannins, and terpenoids may undergo oxidation, hydrolysis, or isomerization during extraction, thereby altering the observed bioactivity [16]. Consequently, the biological activity of an extract reflects the combined effects of its metabolite profile, synergistic or antagonistic interactions among co-extracted compounds, and matrix effects, rather than a simple polarity match [15]. Comparative extraction using polar and non-polar solvents is therefore necessary to identify the extraction system that yields the most favorable balance between metabolite recovery, compound stability, and antioxidant and antibacterial efficacy for this variety.

Earlier work on *M. indica* L. var. madu leaves has dealt mainly with antioxidant activity. Much less is known about whether these leaves also have antibacterial properties and how the choice of extraction solvent affects the compounds obtained. This study therefore examined both antioxidant and antibacterial activities of honey mango leaf extracts. Methanol and n-hexane were used to represent polar and non-polar solvents, and the extracts were tested at several concentrations against *S. aureus* and *E. coli*. Phytochemical screening, DPPH IC₅₀ measurement, and inhibition zone tests were carried out together to give a broader picture of this less-studied variety.

The study aimed to identify the classes of secondary metabolites present in the methanol and n-hexane extracts, determine their antioxidant activity using the DPPH method, and evaluate their antibacterial activity against *S. aureus* and *E. coli* at various concentrations by the disc diffusion method. It also compared how solvent type, extract concentration, and bacterial strain influenced inhibition zone diameters. The findings are expected to support the traditional use of honey mango leaves and to indicate whether

they have potential as natural antioxidants and antibacterial agents for pharmaceutical and nutraceutical applications.

2. MATERIALS AND METHODS

Plant material and sample preparation

Fresh mature leaves of *M. indica* L. var. madu were collected from Sugihwaras Village, Deket Sub-district, Lamongan Regency, East Java, Indonesia (geographical coordinates: 7°00'31.81"S 112°25'36.24"E). The leaves were washed thoroughly with running water to remove dirt and impurities, then dried at room temperature in a shaded area away from direct sunlight. After complete drying, the leaves were ground into powder using a blender to increase the surface area for optimal extraction.

Extraction

Extraction was performed by simple maceration at room temperature with periodic stirring. Dried leaf powder was extracted separately with two solvents. For the methanol extract, 135.01 g of dried leaf powder was macerated in 1.5 L of methanol (polar). For the n-hexane extract, 75.14 g of dried leaf powder was macerated in 1.5 L of n-hexane (non-polar). Each mixture was placed in a glass container covered with aluminum foil. The mixtures were stirred periodically every 24 hours at room temperature for 3 days. After maceration, the mixtures were filtered using Whatman filter paper. The residues were re-macerated twice with fresh solvent to ensure complete extraction. The combined filtrates from each solvent were concentrated using a rotary evaporator at 40 °C and 60 rpm under reduced pressure to obtain crude extracts. The resulting methanol and n-hexane extracts were weighed and stored in sterile vials at 4°C until further analysis. The yield percentage was calculated using the formula:

$$\text{Yield (\%)} = \left(\frac{\text{weight of extract}}{\text{weight of dried sample}} \right) \times 100\%$$

Phytochemical screening

Qualitative phytochemical screening was conducted to detect the presence of secondary metabolites including alkaloids, flavonoids, tannins, saponins, and steroids/terpenoids following standard procedures.

Alkaloid detection: Extract (3 mL) was mixed with 5 mL of 2N HCl and heated for 3 minutes. After cooling, the solution was divided into three test tubes and treated with Mayer, Wagner, and Dragendorff reagents, respectively (2 drops each). Alkaloid presence was indicated by white/yellow precipitate (Mayer), brown precipitate (Wagner), or orange precipitate (Dragendorff) [17].

Flavonoid detection: Extract (1 mL) was mixed with 0.5 mL concentrated HCl and 4 pieces of magnesium ribbon, then heated in a water bath for 15 minutes. A positive result was indicated by the formation of a red, green, or yellow color [18].

Tannin detection: Extract (1 mL) was dissolved in 10 mL distilled water and treated with 4 drops of 10% FeCl₃ solution. A positive result was indicated by dark green or blue-black coloration [19].

Saponin detection: Extract (1 mL) was mixed with 10 mL hot distilled water (40°C) and 2 drops of concentrated HCl, then shaken vigorously for 3 minutes. Saponin presence was indicated by stable foam formation lasting for at least 1 minute [20].

Steroid/Terpenoid detection: Extract (2 mL) was mixed with 0.5 mL chloroform, then 0.5 mL acetic anhydride and 2 drops of concentrated H₂SO₄ were added. A reddish-brown ring indicated terpenoids, while a blue-green ring indicated steroids [21].

Antioxidant activity assay

Antioxidant activity was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay [22]. A DPPH stock solution (1000 ppm) was prepared by

dissolving 1 mg DPPH in 1 mL methanol. This stock was diluted with methanol to obtain a 50 ppm working solution (0.5 mL stock in 10 mL final volume). Sample stock solutions (1000 ppm) were prepared by dissolving 1 mg extract in 1 mL methanol. Five concentrations (10, 20, 30, 40, and 50 µg/mL) were prepared by serial dilution. Each concentration (200 µL) was mixed with 100 µL of DPPH solution (50 ppm) in 96-well microplates. Each concentration was tested in triplicate (n = 3), and the blank and positive control (silymarin) were also assayed in triplicate. The mixtures were incubated in the dark for 1 hour, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer. A blank consisting of DPPH solution in methanol was used as a control, and silymarin served as a positive control. The percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \left| \frac{(\text{Absorbance blank} - \text{absorbance sample})}{\text{Absorbance blank}} \right| \times 100\%$$

IC₅₀ values (the concentration required to scavenge 50% of DPPH radicals) were determined from linear regression equations (y = ax + b). Antioxidant activity was classified according to Pangestu *et al.* [23]: very strong (< 50 µg/mL), strong (50-100 µg/mL), moderate (100-150 µg/mL), weak (150-200 µg/mL), and very weak (> 200 µg/mL).

Bacterial strains and preparation

Staphylococcus aureus (Gram-positive) and *Escherichia coli* (Gram-negative) bacterial strains were obtained from the Microbiology Laboratory collection, Department of Biology, Universitas Airlangga. Bacterial cultures were confirmed macroscopically and microscopically using selective media Manitol Salt Agar (MSA) for *S. aureus* ATCC 25923 and Eosin Methylene Blue Agar (EMB) for *E. coli* ATCC 25922 and Gram staining. For antibacterial testing, bacterial suspensions were prepared in Mueller-Hinton Broth (MHB) and incubated at 37°C for 24 hours. The turbidity was adjusted to 0.5 McFarland

standard (approximately 1.5×10^8 CFU/mL) corresponding to an optical density of 0.1 at 600 nm [24].

Antibacterial activity assay

The antibacterial activity was performed using pathogen such as *Staphylococcus aureus* and *Escherichia coli*. The study evaluated the antibacterial efficiency of *M. indica* var. madu leaves extract against bacterial.

Approximately a 100 of overnight bacterial culture were swabbed separately on sterile Mueller-Hinton Agar (MHA) plates and poured the test sample separately in each discs containing 25 μ L of sample at different concentrations

The extract concentrations of *M. indica* var. madu 6.25%, 12.5%, 25%, and 50% were dissolved in DMSO (10%) followed by serial dilution [25].

Sterile paper discs (6 mm diameter) were impregnated with 25 μ L of each extract concentration and placed on the agar surface. Positive control discs containing 30 μ L of chloramphenicol (1 mg/mL) were used, while 10% DMSO served as a negative control. Plates were incubated at 37°C for 24 hours. The zone of inhibition was recorded using digital calipers with 0.01 mm precision. Each treatment was measured three times within the same experimental unit (technical replicates, $n = 3$), and the mean value was used for analysis. Antibacterial activity was categorized based on Elgaya et al. [26]: strong (> 11 mm), moderate (6-11 mm), and weak (< 6 mm).

Statistical analysis

Data were analyzed using SPSS software version 26. This study employed a completely randomized factorial design with three factors: solvent type (methanol and n-hexane), extract concentration (6.25%, 12.5%, 25%, and 50%), and bacterial species (*Staphylococcus aureus* and *Escherichia coli*), resulting in a $2 \times 4 \times 2$ factorial arrangement. Each treatment combination was performed

in triplicate, and the inhibition zone diameter was used as the response variable.

Normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was assessed using Levene's test. The n-hexane extract produced no inhibition zones at all concentrations, resulting in zero variance; therefore, these data were reported descriptively and excluded from parametric analysis.

For the effect of concentration, the data were normally distributed but did not meet the homogeneity of variances assumption. Therefore, Brown-Forsythe robust ANOVA was used, followed by the Games-Howell post-hoc test. For the effect of solvent type and the combined effect of solvent type and concentration, the data were not normally distributed. Therefore, the Mann-Whitney U test was used to compare solvent types, and the aligned rank transform (ART) ANOVA was used to analyze the combined effect of solvent type and concentration. A p-value < 0.05 was considered statistically significant

3. Results

Maceration of 135.01 g of dried *M. indica* L. var. madu leaves with methanol yielded 27.3 g of methanol extract (20.22% yield), while maceration of 75.14 g of dried leaves with n-hexane yielded 1.3 g of n-hexane extract (1.73% yield). The methanol extract appeared dark green with a thick consistency, whereas the n-hexane extract appeared yellowish-green with a dry consistency (Table 1).

Phytochemical screening revealed distinct secondary metabolite profiles between the two extracts (Table 2). The methanol extract tested positive for alkaloids, flavonoids, tannins, and saponins, whereas the n-hexane extract tested positive only for steroids. Both extracts showed negative results for terpenoids.

The DPPH radical scavenging assay showed that both extracts exhibited concentration-dependent activity (Figure 1). The methanol extract showed very strong antioxidant activity with an IC_{50} of 33.48 ± 0.26 $\mu\text{g/mL}$, which was comparable to silymarin (25.82 ± 0.33 $\mu\text{g/mL}$). In contrast, the n-hexane extract produced less than 20% inhibition at the highest tested concentration (50 $\mu\text{g/mL}$); therefore, its IC_{50} could not be reliably determined and was reported as >50 $\mu\text{g/mL}$ (very weak). A summary of these results is presented in Table 3.

Antibacterial testing using the disc diffusion method revealed that the methanol extract produced inhibition zones against both *Staphylococcus aureus* and *Escherichia coli*, whereas the n-hexane extract showed no activity at any concentration. The overall mean inhibition zone diameters for the two solvents are presented in Table 4. The methanol extract produced a mean inhibition zone of 10.70 ± 2.95 mm, which was significantly different from the n-hexane extract (0.00 ± 0.00 mm) based on the Kruskal-Wallis test ($p < 0.05$).

The effect of extract concentration on the methanol extract is shown in Table 5. The

inhibition zone increased with increasing concentration. The lowest concentration (6.25%) produced 7.78 ± 0.90 mm, while the highest concentration (50%) produced 14.53 ± 1.14 mm. The Games-Howell post-hoc test showed that the 25% and 50% concentrations were significantly different from the 6.25% and 12.5% concentrations ($p < 0.05$). In contrast, the n-hexane extract produced no inhibition zones at all tested concentrations (6.25%, 12.5%, 25%, and 50%), as shown in Table 6.

The overall comparison between the two bacterial species is presented in Table 7. The mean inhibition zone against *S. aureus* was 5.89 ± 6.60 mm, while against *E. coli* it was 4.81 ± 5.47 mm. The Kruskal-Wallis test indicated no significant difference between the two bacterial species ($p > 0.05$).

The combined effects of solvent type, extract concentration, and bacterial species are summarized in Table 8. The highest inhibition zone (15.50 ± 0.26 mm) was observed for the methanol extract at 50% concentration against *S. aureus*. All n-hexane treatments produced no inhibition zones (0.00 ± 0.00 mm). The Mann-Whitney test indicated significant differences among the combined treatments ($p < 0.05$).

Table 1. Extraction results of *M. indica* L. var. madu leaves

Extract	Weight extract (g)	Color	Consistency	Yield (%)
Methanol	27.3	Dark green	Thick	20.22
n-Hexane	1.3	Yellowish-green	Dry	1.73

Table 2. Phytochemical screening results of *M. indica* L. var. madu leaf extracts

Extract	Secondary metabolites					
	Alkaloids	Flavonoids	Tannins	Saponins	Steroids	Terpenoids
Methanol	+	+	+	+	-	-
n-Hexane	-	-	-	-	+	-

Note: (+) = present, (-) = absent

Table 3. IC_{50} values of DPPH radical scavenging activity of *M. indica* L. var. madu extracts

Sample	IC_{50} ($\mu\text{g/mL}$)	Classification
Methanol extract	33.48 ± 0.26	Very strong
n-Hexane extract	Not determined (>50)	Very weak
Silymarin (control)	25.82 ± 0.33	Very strong

The IC_{50} of the n-hexane extract could not be reliably determined because the highest tested concentration (50 $\mu\text{g/mL}$) produced less than 20% DPPH inhibition. Therefore, the IC_{50} is reported as > 50 $\mu\text{g/mL}$ and classified as very weak.

Table 4. Mean inhibition zone diameters of methanol and n-hexane extracts of *Mangifera indica* L. var. *madu* leaves.

Solvent	Mean inhibition zone diameter (mm)
Methanol	10.70 ± 2.95^b
n-Hexane	0.00 ± 0.00^a

Values followed by different superscript letters indicate significant differences among treatments based on the Kruskal-Wallis test ($p < 0.05$)

Table 5. Mean inhibition zone diameters of methanol extract at different concentrations

Concentration (%)	Mean inhibition zone diameter (mm)
6.25	7.78 ± 0.90^a
12.5	9.26 ± 1.27^a
25	11.22 ± 1.78^b
50	14.53 ± 1.14^b

Values followed by different superscript letters indicate significant differences among treatments based on the Games-Howell post-hoc test ($p < 0.05$)

Table 6. Mean inhibition zone diameters of n-hexane extract at different concentrations

Concentration (%)	Mean inhibition zone diameter (mm)
6.25	0.00 ± 0.00
12.5	0.00 ± 0.00
25	0.00 ± 0.00
50	0.00 ± 0.00

No inhibition zones were observed at any concentration

Table 7. Mean inhibition zone diameters against *S. aureus* and *E. coli*.

Bacterial species	Mean inhibition zone diameter (mm)
<i>S. aureus</i>	5.89 ± 6.60^a
<i>E. coli</i>	4.81 ± 5.47^a

The Kruskal-Wallis test showed no significant difference between the two bacterial species ($p > 0.05$)

Table 8. Mean inhibition zone diameters based on solvent type, bacterial species, and extract concentration.

Treatment	Mean inhibition zone diameter (mm)
S1C1B1	8.47 ± 0.18^b
S1C1B2	7.10 ± 0.79^b
S1C2B1	10.37 ± 0.12^c
S1C2B2	8.15 ± 0.55^b
S1C3B1	12.75 ± 0.62^d
S1C3B2	9.68 ± 0.68^c

S1C4B1	15.50 ± 0.26 ^e
S1C4B2	13.57 ± 0.59 ^d
S2C1B1	0.00 ± 0.00 ^a
S2C1B2	0.00 ± 0.00 ^a
S2C2B1	0.00 ± 0.00 ^a
S2C2B2	0.00 ± 0.00 ^a
S2C3B1	0.00 ± 0.00 ^a
S2C3B2	0.00 ± 0.00 ^a
S2C4B1	0.00 ± 0.00 ^a
S2C4B2	0.00 ± 0.00 ^a

Values followed by different superscript letters indicate significant differences among treatments based on the Mann-Whitney test ($p < 0.05$).

Legend:

S1: Methanol; S2: n-Hexane; C1: 6.25%; C2: 12.5%; C3: 25%; C4: 50%; B1: *Staphylococcus aureus*; B2: *Escherichia coli*

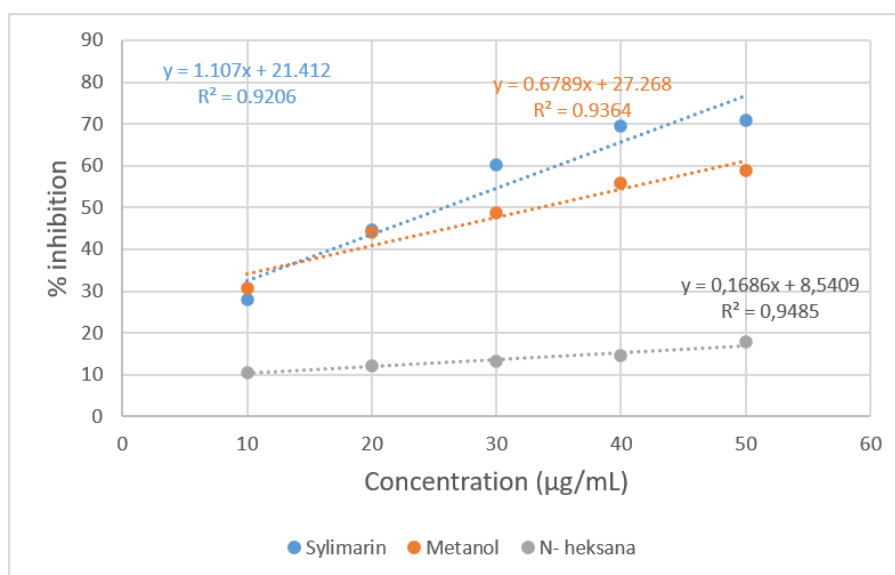


Figure 1. Concentration-response curves for DPPH radical scavenging activity of methanol and n-hexane extracts from *M. indica* L. var. *madu* leaves, compared to silymarin.

4. DISCUSSION

The higher yield obtained from methanol extraction (20.22%) compared to n-hexane extraction (1.73%) indicates that methanol is a more effective solvent for extracting bioactive compounds from *M. indica* L. var. *madu* leaves. This finding aligns with the principle "like dissolves like," where polar solvents extract a wider range of polar to semi-polar secondary metabolites present in plant tissues [13]. Similar results were

reported by Pote and Ipih [27], who found that polar solvents yield higher extract percentages than non-polar solvents due to their ability to dissolve diverse phytochemicals including alkaloids, flavonoids, tannins, and saponins. The maceration method employed in this study is particularly suitable for extracting heat-sensitive compounds as it operates at room temperature, preserving the structural integrity and bioactivity of thermolabile secondary metabolites [28-29]. The physical

characteristics of the extracts, including color and consistency differences, reflect the distinct chemical compositions extracted by each solvent, with the dark green methanol extract indicating the presence of chlorophyll and other polar pigments, while the yellowish-green n-hexane extract suggests the presence of lipophilic compounds [30].

Phytochemical screening revealed a clear distinction in secondary metabolite composition between the two extracts. The methanol extract contained alkaloids, flavonoids, tannins, and saponins, while the n-hexane extract contained only steroids. This differential extraction reflects the polarity-dependent solubility of secondary metabolites. Alkaloids, which contain nitrogen atoms and often exist as salts, are typically soluble in polar solvents [31]. Flavonoids and tannins, characterized by multiple hydroxyl groups, are polar to semi-polar compounds that readily dissolve in methanol [32-33]. Saponins, which possess both hydrophilic sugar moieties and hydrophobic aglycones, are also effectively extracted by polar solvents [34]. In contrast, steroids, which lack polar functional groups, are preferentially extracted by non-polar solvents such as n-hexane [35]. The absence of terpenoids in both extracts, despite the presence of steroids, suggests that the honey mango leaves may contain predominantly steroidal compounds rather than other terpenoids, or that the extraction method was insufficient to isolate specific terpenoid derivatives [36]. Previous studies on honey mango leaves using ethanol extraction reported the presence of both steroids and terpenoids [12], indicating that extraction solvent and method significantly influence the detection of specific compound classes. The absence of alkaloids in the n-hexane extract confirms that non-polar solvents are ineffective for extracting these basic nitrogen-containing compounds, which typically require acidic conditions or polar solvents for efficient recovery [37].

The methanol extract exhibited very strong antioxidant activity with an IC_{50} of 33.48 ± 0.26 $\mu\text{g/mL}$ (mean $\pm$ SD, $n = 3$), whereas the n-hexane extract showed very weak activity and its IC_{50} could not be reliably determined within the tested concentration range ($IC_{50} > 50$ $\mu\text{g/mL}$). The small standard deviation of the methanol extract indicates good reproducibility among replicates. The stronger activity of the methanol extract may be associated with the presence of several classes of polar phytochemicals detected by qualitative screening, such as alkaloids, flavonoids, tannins, and saponins, which are well recognized for their potent radical scavenging activity [38-39]. Flavonoids act as antioxidants through hydrogen atom donation to free radicals, forming stable flavonoid radicals through resonance stabilization [40]. Similarly, tannins with their multiple hydroxyl groups effectively neutralize free radicals through electron transfer mechanisms [41]. Alkaloids, despite being less potent than phenolic compounds, also contribute to antioxidant activity through their nitrogen atoms that can participate in radical scavenging [38,42]. Saponins further enhance antioxidant activity by scavenging superoxide radicals and preventing oxidative damage [43]. The weak antioxidant activity of the n-hexane extract, which contained only steroids, is consistent with the limited radical scavenging capacity of lipophilic compounds. Steroids lack the extensive hydroxylation patterns characteristic of phenolic antioxidants, reducing their ability to donate hydrogen atoms or electrons to neutralize free radicals [44]. Additionally, steroids are primarily composed of hydrocarbon rings with limited functional groups capable of participating in redox reactions, resulting in their classification as weak antioxidants [45]. The IC_{50} value of 33.48 $\mu\text{g/mL}$ obtained in this study is lower (indicating stronger activity) than the previously reported value of 79.73 ppm for the ethanol extract of *M. indica* L.

var. *madu* leaves [12], suggesting that methanol is a more effective solvent for extracting antioxidant compounds from this variety. This superiority may be attributed to methanol's higher polarity compared to ethanol, allowing more efficient extraction of phenolic compounds [46]. The comparable antioxidant activity of the methanol extract to silymarin ($IC_{50} = 25.82 \pm 0.33 \mu\text{g/mL}$), a well-established hepatoprotective agent with potent antioxidant properties [47], further validates the extract's potential as a natural antioxidant source.

The methanol extract demonstrated concentration-dependent antibacterial activity against both tested bacterial strains, while the n-hexane extract showed no activity. The methanol extract produced a mean inhibition zone of $10.70 \pm 2.95 \text{ mm}$, whereas the n-hexane extract produced $0.00 \pm 0.00 \text{ mm}$. This difference was statistically significant based on the non-parametric test used for solvent comparison ($p < 0.05$; Table 4). This differential activity can be directly correlated with the phytochemical composition: the methanol extract contains multiple antibacterial compounds with diverse mechanisms of action, whereas the n-hexane extract contains only steroids, whose antibacterial activity is limited to membrane disruption. Alkaloids exert antibacterial effects through several mechanisms, including interference with peptidoglycan synthesis, inhibition of protein synthesis, and disruption of nucleic acid metabolism [48], [49]. Flavonoids and tannins, both belonging to the phenolic compound class, act by compromising cell membrane integrity, inhibiting essential enzymes, and damaging protein and DNA structures [32]. Specifically, flavonoids have been shown to form complexes with bacterial cell walls and membranes, increasing permeability and causing leakage of cellular contents [50]. Tannins inhibit bacterial growth by binding to and precipitating proteins, thereby inactivating enzymes involved in cell wall synthesis and disrupting membrane transport

systems [51]. Saponins, with their amphiphilic nature, interact with and disrupt bacterial cell membranes, leading to cell lysis and death [52-53].

The effect of extract concentration was statistically significant based on Brown-Forsythe robust ANOVA ($F = p < 0.05$). The Games-Howell post-hoc test showed that the 25% and 50% concentrations produced significantly larger inhibition zones than the 6.25% and 12.5% concentrations ($p < 0.05$). The inhibition zone increased from $7.78 \pm 0.90 \text{ mm}$ at 6.25% to $14.53 \pm 1.14 \text{ mm}$ at 50%. This concentration-dependent response follows established principles of antimicrobial action, where higher concentrations result in increased compound diffusion and enhanced interaction with bacterial cells [56]. The highest antibacterial activity ($15.50 \pm 0.26 \text{ mm}$) was observed for the methanol extract at 50% concentration against *S. aureus*. This activity level falls within the "strong" category ($>11 \text{ mm}$) according to Elgayyar et al. [26], indicating significant antibacterial potential. However, the activity was substantially lower than chloramphenicol (39.53 mm), which is expected given that chloramphenicol is a purified antibiotic with specific, potent mechanisms targeting protein synthesis, while the methanol extract is a crude mixture containing multiple compounds with varying concentrations and activities [57]. The absence of antibacterial activity in the n-hexane extract at all tested concentrations reinforces the conclusion that non-polar extracts from *M. indica* L. var. *madu* leaves lack sufficient bioactive compounds to inhibit bacterial growth. This finding is consistent with studies on other plant species where polar extracts generally exhibit superior antibacterial activity due to the presence of phenolic compounds and other polar bioactive molecules [58-59].

The observation that the methanol extract exhibited antibacterial activity against both *S. aureus* (Gram-positive) and *E. coli* (Gram-negative), with no significant difference between them based on the

Kruskal-Wallis test ($p > 0.05$; Table 7), indicates broad-spectrum activity. The mean inhibition zone against *S. aureus* was 5.89 ± 6.60 mm, while against *E. coli* it was 4.81 ± 5.47 mm. This is noteworthy because Gram-negative bacteria possess an outer membrane containing lipopolysaccharides that serve as an additional permeability barrier, often reducing susceptibility to antimicrobial compounds [54-55]. The lack of a significant difference suggests that the active compounds in the methanol extract can effectively penetrate the outer membrane of *E. coli*, likely through disruption of lipopolysaccharide structure or porin channels, reaching their intracellular targets and exerting antibacterial effects comparable to those against Gram-positive bacteria. However, because the overall means include the zero values from the n-hexane treatments, the species comparison should be interpreted with caution; when only the methanol extract is considered, *S. aureus* tended to be more susceptible than *E. coli* at each concentration.

The interaction between solvent type and extract concentration was analyzed using aligned rank transform (ART) ANOVA, which showed a significant interaction ($F = p < 0.05$; Table 8). This indicates that the effect of concentration depended on the solvent used. Because the n-hexane extract produced no inhibition zones at any concentration, this interaction was driven mainly by the concentration-dependent activity of the methanol extract. The combined treatment results in Table 8 confirm that the highest inhibition zone (15.50 ± 0.26 mm) was observed for the methanol extract at 50% concentration against *S. aureus*, while all n-hexane treatments produced no inhibition zones (0.00 ± 0.00 mm). These results confirm that antibacterial efficacy is determined not by solvent type or concentration alone, but by the combination of both factors.

The results of this study underscore the critical role of solvent selection in

determining the bioactivity of plant extracts. The superior performance of methanol across both antioxidant and antibacterial assays can be attributed to its ability to extract a broader and more diverse range of secondary metabolites, including those with established antioxidant and antibacterial properties. This finding aligns with the “like dissolves like” principle: polar compounds required for bioactivity are preferentially extracted by polar solvents [13-14]. The disparity between methanol and n-hexane extracts in this study contrasts with some previous reports where non-polar extracts exhibited bioactivity [60]. This discrepancy may be due to differences in plant variety, environmental conditions, or specific compound profiles. For example, while n-hexane extracts of some mango varieties may contain significant levels of bioactive terpenoids or other lipophilic antioxidants, the *M. indica* L. var. *madu* variety in this study appears to have predominantly polar bioactive compounds, resulting in negligible non-polar extract bioactivity [58-61].

The very strong antioxidant activity and moderate-to-strong antibacterial activity of the methanol extract from *M. indica* L. var. *madu* leaves position this extract as a promising candidate for pharmaceutical and nutraceutical development. The extract could potentially serve as a natural preservative in food products, an ingredient in functional foods or dietary supplements for oxidative stress management, or as a lead for developing novel antibacterial agents to combat antibiotic-resistant infections [7-8]. The broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria is particularly significant given the current antibiotic resistance crisis. The ability to effectively inhibit *S. aureus* and *E. coli* suggests that the extract may contain compounds or combinations of compounds that overcome common resistance

mechanisms. Future studies should focus on isolating and identifying individual bioactive compounds responsible for the observed activities, evaluating their mechanisms of action, and testing efficacy against clinically relevant antibiotic-resistant strains.

Several limitations of this study should be acknowledged. First, the n-hexane extract produced zero inhibition zones at all concentrations, resulting in zero variance; therefore, these data could not be included in parametric analysis and were reported descriptively. Second, the antibacterial activity was assessed using in vitro methods, which may not fully predict in vivo efficacy due to factors such as bioavailability, metabolism, and immune system interactions. Third, the extracts were crude mixtures, making it difficult to attribute activity to specific compounds. Fourth, the study evaluated only two bacterial strains; broader testing against a panel of pathogens would strengthen the evidence. Fifth, the interaction between solvent type and bacterial species, as well as concentration and bacterial species, was not statistically tested; future studies using a full factorial model or ART ANOVA should examine these interactions. Future research directions should include: (1) bioassay-guided fractionation to isolate and identify the active compounds responsible for antioxidant and antibacterial activities; (2) in vivo studies to evaluate safety and efficacy; (3) formulation development for practical applications; and (4) investigation of the extract's potential to

enhance antibiotic activity through synergistic combinations with conventional antibiotics.

5. CONCLUSION

This study provides the first comprehensive evaluation of secondary metabolites, antioxidant activity, and antibacterial activity from *Mangifera indica* L. var. *madu* (honey mango) leaves using methanol and n-hexane extracts. The methanol extract, which contained alkaloids, flavonoids, tannins, and saponins, exhibited very strong antioxidant activity (IC_{50} = 33.48 μ g/mL) and concentration-dependent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*, with the highest activity observed at 50% concentration (15.50 mm against *S. aureus*). The n-hexane extract, which contained only steroids, showed very weak antioxidant activity (IC_{50} = 245.90 μ g/mL) and no antibacterial activity. Solvent type was a determining factor, with methanol extraction proving far superior to n-hexane for recovering bioactive compounds. Bacterial strain did not significantly affect activity, indicating broad-spectrum antibacterial potential. These findings establish *M. indica* L. var. *madu* leaves as a promising source of natural antioxidants and antibacterial agents, supporting their traditional use and providing a scientific basis for further development into pharmaceutical or nutraceutical products

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