ORIGINAL ARTICLE

Antibacterial Susceptibilities of *Moringa Oleifera* Leaf Extract (MOLE) Against Gram-positive and Gram-negative Bacteria: A Preliminary Study

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ABSTRACT

Introduction: The emergence of antibiotic-resistant bacteria, particularly Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA), along with methicillin-susceptible *S. aureus* (MSSA) and Gram-negative *Escherichia coli* (*E. coli*), highlights the critical need for new antibacterial approaches. Herbal medicines, particularly *Moringa oleifera* leaf extract (MOLE), have demonstrated potential in combating bacterial infections. This study assesses the antibacterial efficacy of MOLE against MRSA, MSSA, and *E. coli*, with a focus on its effectiveness against both resistant and non-resistant bacterial strains. **Methods:** The antimicrobial susceptibility of ethanolic extract of MOLE was evaluated using the Kirby-Bauer disk diffusion method at five different concentrations (50-800 mg/mL) against MRSA, MSSA and *E. coli*. The phytochemical composition of MOLE was analysed using liquid chromatography-mass spectrometry (LC-MS). **Results:** MOLE demonstrated dose-dependent antibacterial susceptibility against both MSSA and MRSA. For MSSA, inhibition zones increased from 11.40 ± 0.65 mm at 100 mg/mL to 21.67 ± 0.75 mm at 800 mg/mL. MRSA exhibited similar dose-response, with inhibition zones expanding from 9.33 ± 0.65 mm at 100 mg/mL to 17.00 ± 0.65 mm at 800 mg/mL, comparable to the positive control, cefoxitin (18.75 ± 0.65 mm). Notably, MOLE exhibited no inhibitory effect against *E. coli*. LC-MS analysis identified bioactive compounds, including flavonoids, alkaloids, phenolics, and glucosinolates, known for their antibacterial properties. **Conclusion:** MOLE exhibited significant antimicrobial susceptibility against MRSA and MSSA, but was ineffective against *E. coli*. Future research should aim to elucidate the mechanisms of action of MOLE, evaluate its safety and efficacy *in vivo*, and explore potential synergistic interactions with conventional antibiotics.
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INTRODUCTION

In the face of escalating global health threats posed by antibiotic-resistant bacteria, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), traditional antibiotics are increasingly ineffective, necessitating the exploration of alternative therapeutic strategies (1). MRSA infections are notorious for their resistance to multiple antibiotics, which complicates treatment regimens and increases the risk of severe outcomes in clinical settings worldwide (2). In 2024, the World Health Organisation (WHO) revised its Bacterial Priority Pathogens List (BPPL), placing methicillin-resistant *Staphylococcus aureus* (MRSA) in the 14th position among bacteria that pose the greatest threat to human health as a result of antibiotic resistance (3). The emergence and persistence of MRSA highlight a critical need for innovative approaches to combat this resilient pathogen. Alongside MRSA, methicillin-susceptible *S. aureus* (MSSA) and Gram-negative pathogens like *Escherichia coli* (*E. coli*) also pose considerable therapeutic obstacles, underscoring the necessity for novel antimicrobial agents (4, 5). *E. coli* can be disseminated via contaminated food and water, with cattle serving as a notable reservoir (6). The rising resistance of *E. coli*, particularly in extended-spectrum beta-lactamase (ESBL)-producing strains, exacerbates treatment and control challenges (7). The National Antibiotic Resistance Surveillance Report 2023 indicates that both MRSA and *E. coli* have exhibited escalating resistance to multiple antibiotics. MRSA has shown heightened resistance to erythromycin, rifampicin, and

fusidic acid, while *E. coli* has demonstrated increasing resistance to ceftazidime, cefotaxime, and carbapenems, although resistance rates to imipenem and meropenem have slightly decreased (8).

The urgency to address antibiotic resistance has prompted extensive research into natural products as potential sources of new antimicrobial agents. *Moringa oleifera* (*M. oleifera*), commonly known as the drumstick tree, stands out within this realm for its comprehensive phytochemical profile and documented biological activities (9). Originating from subtropical and tropical regions, *M. oleifera* has been utilized for centuries in treating a wide array of ailments, owing to its diverse pharmacological properties, including anti-inflammatory, antioxidant, and antimicrobial effects (10). The plant's leaves, in particular, contain bioactive compounds such as flavonoids, alkaloids, phenolic acids, and glucosinolates, which have been studied for their potential therapeutic benefits (11).

In particular, the antibacterial activity of *M. oleifera* extracts has been reported against a wide range of pathogens, including both Gram-positive and Gram-negative bacteria (12). Alkaloids found in *M. oleifera*, such as moringine and spirochin, have shown promising antimicrobial properties by disrupting bacterial cell walls and inhibiting essential enzymes (13). Flavonoids, another group of compounds abundant in *M. oleifera*, exhibit antioxidant and antimicrobial effects, potentially enhancing the plant's therapeutic efficacy against resistant bacteria (14).

Despite the promising attributes of *M. oleifera*, there remains a significant gap in the literature concerning its specific effectiveness against methicillin-resistant and methicillin-susceptible strains of *S. aureus* (MSSA). Furthermore, while research has predominantly focused on the antibacterial activity of *M. oleifera* leaf extract (MOLE) against various Gram-negative bacteria, there is a limited investigation into its effectiveness specifically against *E. coli* (15). It is essential to address these gaps in order to fully comprehend the potential of *M. oleifera* as a versatile antimicrobial agent and its effectiveness in treating a wide range of bacterial infections.

In light of these considerations, the present study aims to contribute to filling this gap by investigating the antibacterial effects of MOLE against MRSA, MSSA and *E. coli*. By employing standardized methods to evaluate MOLE's efficacy, this research seeks to provide valuable insights into its potential as a natural alternative or adjunct therapy for combating antibiotic-resistant *S. aureus* infections and also assess its effectiveness against *E. coli*. Understanding the MOLE's activity across different bacterial strains is crucial for optimizing its therapeutic applications and supporting the development of new natural treatment options.

Materials and Methods

Sample Preparation and Extraction

M. oleifera leaves (Figure 1) were collected at Kg. Tun Razak, Bukit Katil, Melaka, Malaysia. The plant sample of *M. oleifera* was sent for verification at the Biodiversity Unit, Institute of Bioscience, University Putra Malaysia and authenticated with voucher number KM 0088/23, which was done through morphological assessment and comparison with reference specimens.

Extraction of *M. oleifera* leaf extract (MOLE) was performed using maceration techniques with 80% ethanol as the solvent, following established protocols with slight modifications (16). Briefly, 100 grams of *M. oleifera* leaf powder was macerated in 1 litre of 80% ethanol for 72 hours at room temperature with occasional shaking. The extract was filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure using a rotary evaporator (Buchi Rotavapor R200, Switzerland) at 60°C. The concentrated extract was subsequently lyophilized to obtain a dried powder form of MOLE. The yield of the extract was determined gravimetrically and stored at -20°C until further analysis. This extraction process was performed in triplicates. The percentage yield of MOLE is 20.78%. The extraction yield (%) was calculated as follows:

$$\text{Extraction yield}(\%) = \frac{(\text{Weight of concentrated extract (g)}) \times 100}{(\text{Weight of dried plant sample (g)})}$$

Figure 1: *M. oleifera* leaves. Leaves were collected at Bukit Katil, Melaka, showcasing the characteristic morphology and green colouration of the plant.

Liquid Chromatography–Mass Spectrometry (LC-MS) Analysis of MOLE

The active compounds in MOLE were determined using Liquid Chromatography–Mass Spectrometry (LC-MS). 1 mg/mL of MOLE extract was prepared in methanol and filtered through the 0.22 µm PTFE membrane filter prior to analysis. The analysis was completed using Agilent 1290 Infinity LC system coupled to Agilent 6520 Accurate-Mass Q-TOF (Agilent Technologies Inc., California, United States) with dual, positive and negative ionization mode (ESI). The mass ranges for both positive and negative ion polarity were 100-1000 m/z and 115-1000 m/z, respectively. The compounds were analysed using Agilent MassHunter Qualitative Analysis software (Agilent Technologies Inc., California, United States).

Bacterial Strains

Two strains of *S. aureus* [MSSA (Methicillin-Susceptible *Staphylococcus aureus*): ATCC 25923; MRSA (Methicillin-Resistant *Staphylococcus aureus*): ATCC 33591)] and *E. coli* (ATCC 25922) were employed in the study. The strains were obtained from the collection of the Applied Microbiology Laboratory of the University of Putra Malaysia. The identity of the *S. aureus* and *E. coli* strains was confirmed through Gram staining, coagulase and the catalase tests prior to use. The confirmed *S. aureus* and *E. coli* strains were maintained at -30 °C in brain heart infusion (BHI) (Oxoid, UK) broth containing 20% glycerol until further use.

Preparation of Inoculums

Prior to assays, all bacterial strains were maintained in a viable state via inoculation on Trypticase Soy Agar (TSA) (Merck, Germany) and incubated overnight at 37 °C under aerobic conditions.

Kirby-Bauer Disk Diffusion Test

The antimicrobial susceptibility of MOLE against MRSA (ATCC 33591), MSSA (ATCC 25923) and *E. coli* (ATCC 25922) strains was assessed using the Kirby-Bauer disk diffusion method, following Clinical and Laboratory Standards Institute (CLSI 2023) guidelines. Bacterial suspensions were prepared by inoculating overnight cultures into normal saline and adjusting the turbidity to 0.5 McFarland units, corresponding to a cell density of 1.5 x 108 CFU/mL.

Standardized bacterial suspensions were spread onto sterile Mueller-Hinton Agar (MHA) (HiMedia, India) plates using sterile cotton swabs. Commercial blank disks (6.0 mm diameter, Oxoid) were impregnated with MOLE dissolved in distilled water at varying concentrations (50 mg/mL, 100 mg/mL, 200 mg/mL, 400 mg/mL, and 800 mg/mL) and placed aseptically onto the inoculated MHA plates. The plates were left at room temperature for 30 minutes to ensure adequate diffusion before incubation at 37°C for 16-18 hours. Negative controls consisted of disks impregnated with distilled water (0 mg/mL), while positive controls included cefoxitin disks (CFX, 30 µg) (Liofilchem, Italy) per recommendation of CLSI guidelines (17). Each assay was performed in triplicates, and the antimicrobial susceptibility was expressed as the mean inhibition diameter (mm) ± standard error of the mean (SEM).

Statistical Analysis

Statistical analysis was performed using GraphPad PRISM version 10.2.3 statistical analysis software (GraphPad Software, La Jolla California USA). One-way analysis of variance (ANOVA) was used to determine the significance of the antibacterial effect of MOLE at different concentrations of exposure to MRSA and MSSA. Data were presented as the mean ± standard error mean (SEM), and the data were significant when the p-value was ≤0.05.

RESULTS

Phytochemical Analysis of MOLE

LC-MS analysis of MOLE identified a diverse array of bioactive compounds, including alkaloids, flavonoids, phenolics, and glycosides (Table I). The total ion chromatogram (positive mode) and total ion chromatogram (negative mode) are given in Figure 2 and Figure 3, respectively. LC-MS characterization of MOLE revealed the presence of nearly 45 compounds. Out of these 45 compounds, 14 compounds were predicted to possess antibacterial activity based on previous studies. The 14 compounds predicted to possess antibacterial activity include 8-Hydroxyluteolin 8-glucoside, Isovitexin, Quercetin 3- (6''-malonylgalactoside), Apigenin 5-glucoside, 3,4-DHPEA-EA, Vanilloloside, (R)- Roemerine, Cepharanthine, Isosyringinoside, Davallioside A, Orobol 7-O-(6''- malonylglucoside), Desulfoglucotropeolin, Phytosphingosine, Emmotin A. The phytochemical analysis for these 14 compounds is shown in Table I.

Table I: Bioactive Compounds Identified in MOLE with Antibacterial Properties using LC-MS

Compound	Molecular formula	Retention Time (RT)	Molecular mass	m/z	Ion (+/-)	Classification
8-Hydroxyluteolin 8-glucoside	$C_{21}H_{20}O_{12}$	8.979	464.0956	465.103	[M+H]+	Flavonoids
Isovitexin	$C_{21}H_{20}O_{10}$	8.825	432.1061	433.1135	[M+H]+	
Quercetin 3-(6''-malonylgalactoside)	$C_{24}H_{22}O_{15}$	9.241	550.0965	551.104	[M+H]+	
Apigenin 5-glucoside	$C_{21}H_{20}O_{10}$	8.865	432.1074	433.1148	[M+H]+	
3,4-DHPEA-EA	$C_{19}H_{22}O_{8}$	0.732	378.1311	377.1239	[M-H]-	Phenolics
Vanilloloside	$C_{14}H_{20}O_{8}$	2.511	316.1153	315.108	[M-H]-	
(R)-Roemerine	$C_{18}H_{17}NO_{2}$	20.434	279.1266	280.1343	[M+H]+	Alkaloids
Cepharanthine	$C_{37}H_{38}N_{2}O_{6}$	20.492	606.2718	607.2793	[M+H]+	
Isosyringinoside	$C_{23}H_{34}O_{14}$	0.762	534.1951	533.1879	[M-H]-	Glycosides
Davallioside A	$C_{26}H_{32}O_{13}$	1.194	535.1696	570.139	[M-H]-	
Orobol 7-O-(6''-malonylglucoside)	$C_{24}H_{22}O_{14}$	9.625	534.1018	535.1091	[M+H]+	
Desulfoglucotropeolin	$C_{10}H_{17}NO_{8}S$	1.462	329.0925	330.0997	[M+H]+	
Phytosphingosine	$C_{14}H_{19}NO_{6}S$	1.462	317.2937	318.3008	[M+H]+	Lipids
Emmotin A	$C_{16}H_{14}O_{4}$	16.669	278.1516	279.159	[M+H]+	Terpenoids

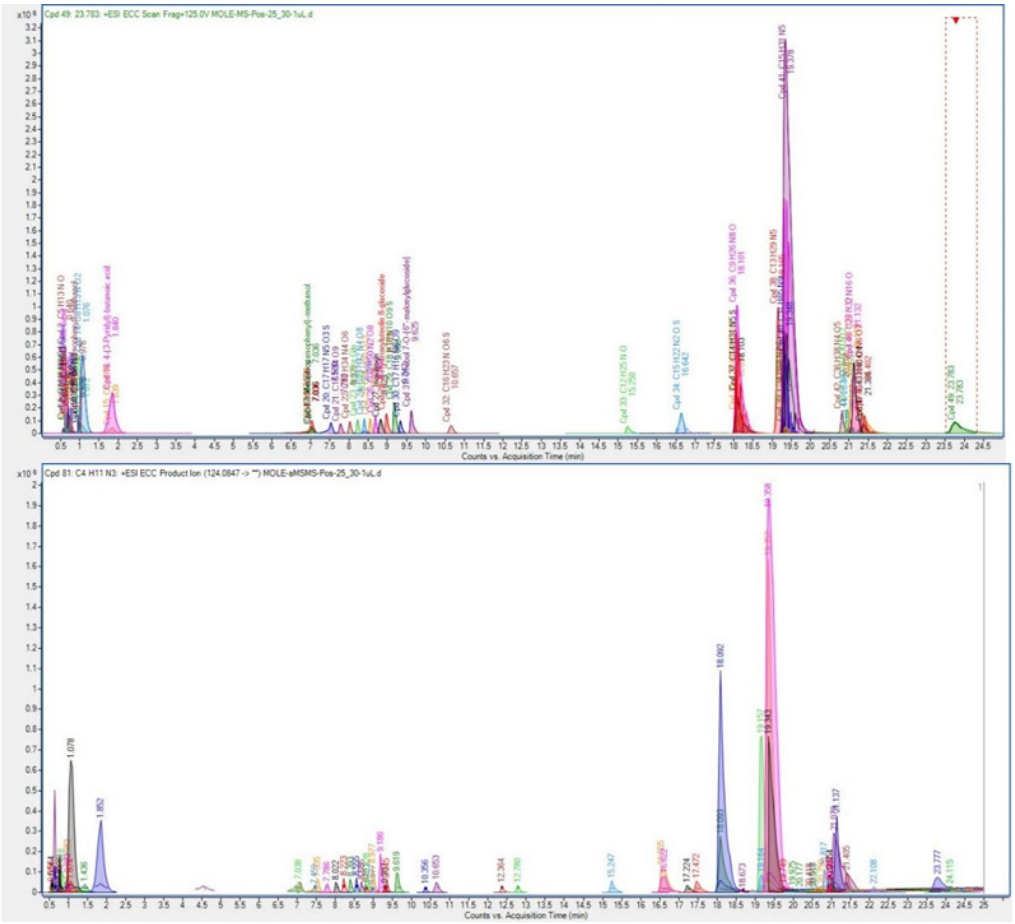

Figure 2: Total ion chromatogram (TIC) in positive ion mode. This chromatogram illustrates the ion intensity profile obtained using positive ionization mode during the sample analysis.

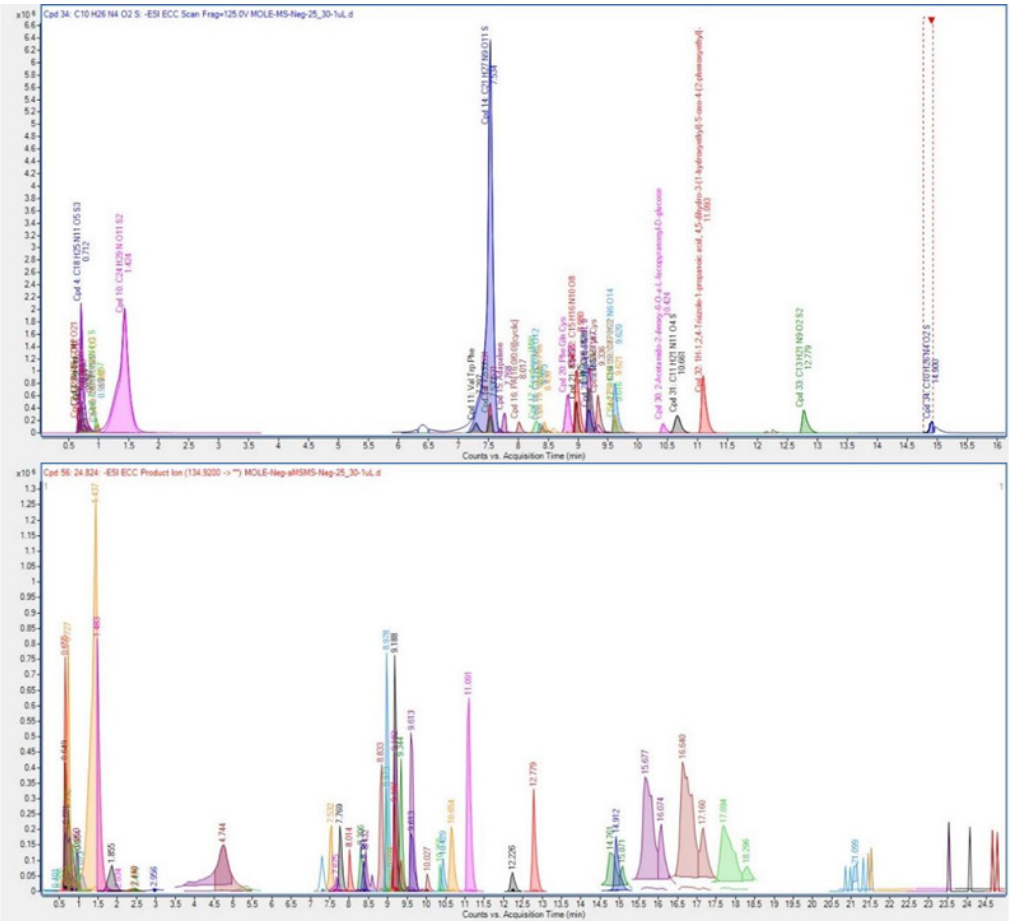

Figure 3: Total ion chromatogram (TIC) in negative ion mode. This chromatogram displays the ion intensity profile captured using negative ionization mode during the sample analysis.

Antimicrobial Susceptibility of MOLE

A one-way ANOVA was performed to determine significant differences in inhibition zones between MOLE concentrations for MRSA and MSSA ($p \leq 0.05$). Statistical significance was denoted in Figure 4. The antibacterial effects of MOLE were investigated against MRSA, MSSA and *E. coli* using the Kirby-Bauer disk diffusion test. MOLE exhibited significant dose-dependent antimicrobial susceptibility against both MRSA and MSSA strains (Figure 4). The mean diameters of inhibition zones increased with higher concentrations of MOLE, indicating enhanced antibacterial efficacy.

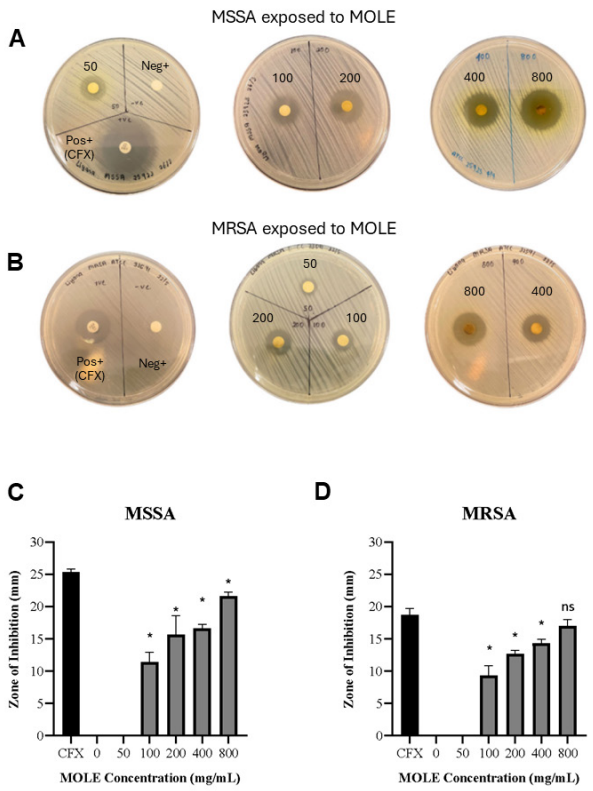

Figure 4: Zone of inhibition of (A&C) MSSA and (B&D) MRSA following exposure to MOLE for 16 hours at different concentrations (50–800 mg/mL). MRSA and MSSA treated with distilled water (0 mg/mL) served as the negative control, while Cefoxitin (CFX) served as the positive control. Data represent mean ± SEM (n = 3-5). Significant differences between experimental groups and the positive control (CFX) are denoted by *, while 'ns' donates not significant, with statistical significance determined using a one-way ANOVA followed by a post-hoc Dunnett's test ($p \leq 0.05$).

The antimicrobial susceptibility of MOLE was evaluated against MSSA using various concentrations (0, 50, 100, 200, 400, and 800 mg/mL). The positive control, cefoxitin (CFX), demonstrated a consistent zone of inhibition with a mean value of 25.33 mm. As expected, the control group (0 mg/mL MOLE) exhibited no antimicrobial susceptibility. At 50 mg/mL, MOLE showed no zone of inhibition, indicating no efficacy at this concentration. However, MOLE at 100 mg/mL displayed a mean zone of inhibition of 11.40 mm. Higher concentrations of MOLE showed increased antimicrobial susceptibility, with mean zones of inhibition of 15.67 mm at 200 mg/mL, 16.67 mm at 400 mg/mL, and 21.67 mm at 800 mg/mL. These results indicate a dose-dependent antibacterial effect of MOLE against MSSA, suggesting that MOLE could be a potent antibacterial agent at higher concentrations.

Similarly, the antimicrobial susceptibility of MOLE was tested against MRSA. CFX served as the positive control and exhibited a mean zone of inhibition of 18.67 mm. The control group (0 mg/mL MOLE) showed no antimicrobial susceptibility. At 50 mg/mL, MOLE did not inhibit MRSA growth. The mean zones of inhibition for MOLE were 9.33 mm at 100 mg/mL, 12.67 mm at 200 mg/mL, and 14.33 mm at 400 mg/mL, indicating a dose-dependent response. At 800 mg/mL, MOLE showed a mean zone of inhibition of 17.00 mm, which was comparable to that of CFX, highlighting MOLE's potential as an antibacterial agent against MRSA. These findings demonstrate that MOLE exhibits significant antimicrobial susceptibility even against antibiotic-resistant strains like MRSA.

In contrast to the substantial activity observed against MRSA and MSSA, MOLE failed to exhibit any antibacterial effect against *E. coli* at any of the tested concentrations (0, 50, 100, 200, 400, 800 mg/mL). The absence of inhibitory zones (Figure 5) suggests that, in these experimental settings, *E. coli* is not susceptible to MOLE.

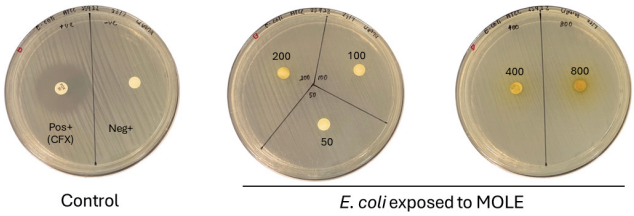

Figure 5: Zone of inhibition of *E. coli* following exposure to MOLE at different concentrations (50–800 mg/mL) for 16 hours. The plates show that no inhibition zones are present at any concentration, indicating that MOLE does not exhibit antibacterial susceptibility against *E. coli*.

DISCUSSION

The investigation into the antibacterial properties of MOLE has yielded promising results, particularly in its activity against MRSA and MSSA. Our findings highlighted several important points regarding the antimicrobial susceptibility of MOLE. Primarily, MOLE exhibited dose-dependent antimicrobial susceptibility against both MRSA and MSSA. Increased concentrations of MOLE correlated with enhanced efficacy in inhibiting bacterial growth, with this effect observed consistently across concentrations ranging from 50 mg/mL to 800 mg/mL. This observation suggests that the antibacterial compounds in MOLE act synergistically to enhance their inhibitory effects at higher concentrations, which aligns with previous studies that have shown dose-dependent antimicrobial activities of plant extracts (17).

The present study demonstrated antimicrobial susceptibility of MOLE against MSSA and MRSA

at concentrations as low as 50 mg/mL. Although antimicrobial susceptibility of MOLE against *S. aureus* have been reported in multiple studies at concentrations ranging from 0.02 to 800 mg/mL (18), to the best of our knowledge, few have reported a comparable zone of inhibition to positive control against methicillin-resistant strains (19, 20). The present study highlights that MOLE achieved a comparable zone of inhibition to the positive control against MRSA at a concentration of 800 mg/mL. This observation indicates that MOLE at the aforementioned concentration exhibited potent antimicrobial susceptibility against resistant strains, which is comparable to a commercial antibiotic, cefoxitin. Given the global health challenge posed by MRSA, MOLE presents a viable alternative or adjunct therapy to traditional antibiotics. The potential of MOLE at high concentrations suggests that its bioactive compounds can effectively combat resistant bacterial strains, as supported by previous research on plant-derived antimicrobials (21).

Interestingly, MRSA showed higher sensitivity to MOLE compared to MSSA. This differential sensitivity could be due to variations in the cell wall structure or efflux pump systems between the two strains, which may influence their susceptibility to MOLE (22, 23). MRSA's higher sensitivity suggests that MOLE or its bioactive compounds might be more effective at targeting the specific mechanisms that MRSA uses to evade conventional antibiotics. Further studies are needed to confirm these findings and explore the underlying mechanisms. Furthermore, previous studies have shown that the genomic analysis of *S. aureus* strains highlights significant genetic diversity between MRSA and MSSA, impacting gene expression related to transport systems and two-component systems (24). These genetic differences could influence the sensitivity to antimicrobial agents, including MOLE. This aligns with the notion that certain plant compounds may target specific bacterial features more effectively (25). The variations in gene expression related to transport and two-component systems in MRSA and MSSA may thus play a crucial role in their distinct responses to MOLE, potentially explaining the observed differences in sensitivity.

In contrast to the positive results obtained against MRSA and MSSA, *E. coli* showed complete resistance to MOLE across all tested concentrations. In the present study, no inhibitory effect on *E. coli* was seen despite testing different concentrations, indicating that MOLE used in this study may demonstrate strain-specific activity or that other factors, such as the method of extraction, could influence its efficacy(26). The resistance of *E. coli* in this present study aligns with a previous study on the antibacterial effects of aqueous and ethanolic extracts of *M. oleifera* leaves, which also showed no activity against *E. coli* as opposed to Gram-positive bacteria (27). This finding contrasts with previous studies, which have demonstrated that *E. coli* is susceptible to MOLE, with extracts of *M. oleifera* exhibiting varying degrees of antimicrobial susceptibility against *E. coli* under diverse conditions. These studies indicate that MOLE's antibacterial activities are efficient against *E. coli*, with factors including extract concentration, bacterial strain, and experimental methods affecting the observed outcomes (28, 29). The absence of inhibitory effects against *E. coli* in this study suggests that MOLE's antibacterial action may be specific to Gram-positive bacteria. These findings most likely stem from the structural differences between Gram-positive and Gram-negative bacteria. Gram-negative bacteria, like *E. coli*, have an additional outer membrane comprised of lipopolysaccharides, which can serve as a barrier, restricting the entry of MOLE's bioactive compounds (23). The presence of an outer membrane, combined with efflux pumps and other resistance mechanisms typically observed in Gram-negative bacteria, may account for *E. coli*'s resistance to MOLE (30).

Previous studies have demonstrated that plant extracts often exhibit greater efficacy against Gram-positive bacteria due to their simpler cell wall structure, which lacks the outer membrane found in Gram-negative bacteria (31, 32). A recent study on natural cinnamic acid derivatives revealed 17 molecules, seven of which demonstrated significant inhibitory effects on *Escherichia coli* β-glucuronidase. Compounds devoid of a hydrogen atom at R1 or possessing bulky groups at R9 exhibited diminished effectiveness, highlighting the influence of these structural features on antibacterial efficacy (33). The inability of MOLE to inhibit *E. coli* may indicate that its bioactive compounds are less potent against particular molecular targets in Gram-negative bacteria. This inclination suggests that increased concentrations of MOLE or alternate extraction methods may improve its efficacy against these bacteria and warrants investigation in future research. Additionally, combining MOLE with other antimicrobial agents may help overcome the resistance mechanisms of Gram-positive and Gram-negative bacteria, potentially leading to synergistic effects. For instance, a past study revealed that the combination of *M. oleifera* leaf ethanol extract (20-50%) and amoxicillin (3 mg/mL) demonstrated a synergistic antibacterial effect against *S. aureus* and *E. coli* in vitro, as indicated by an inhibition zone diameter that is greater than 17 mm (34).

The lack of activity against *E. coli* does not diminish the potential of MOLE as an antimicrobial agent. Instead, it highlights the importance of tailoring antimicrobial therapies to target specific bacterial types. The focus on Gram-positive pathogens, particularly resistant strains such as MRSA, positions MOLE as a promising candidate for further development as an adjunctive therapy. Moreover, this finding underscores the need to investigate alternative strategies when addressing Gram-negative bacteria, which present a unique set of

challenges in the context of antibiotic resistance.

In the present study, the LC-MS analysis identified several key bioactive compounds in MOLE that likely contribute to its antibacterial properties. Flavonoids such as 8-hydroxyluteolin 8-glucoside, isovitexin, quercetin 3-(6''-malonylgalactoside), and apigenin 5-glucoside are well-known for their antimicrobial and antioxidant activities. These compounds can disrupt bacterial cell walls, inhibit nucleic acid synthesis, and interfere with energy metabolism, enhancing the antibacterial efficacy of MOLE (35, 36).

Phenolic compounds identified include 3,4-DHPEA-EA and vanilloloside. Phenolics are widely recognized for their antimicrobial effects due to their ability to disrupt bacterial cell membranes and inhibit essential enzymes involved in bacterial metabolism (37). Alkaloids such as (R)-Roemerine and cepharanthine are known for their broad-spectrum antimicrobial properties, with both demonstrating antibacterial activity across multiple studies. (R)-Roemerine exhibits activity against *E. coli*, *Bacillus subtilis, Salmonella typhimurium*, and MRSA by influencing outer membrane permeability and suppressing transport proteins involved in carbohydrate metabolism, thereby impairing cellular growth (38). Meanwhile, cepharanthine stabilizes plasma membrane fluidity, disrupting bacterial cell membranes and essential functions like nutrient uptake and waste elimination, ultimately hindering bacterial growth and survival (39). Furthermore, isosyringinoside, Davallioside A, orobol 7-O-(6''-malonylglucoside), and desulfoglucotropeolin categorized under glycosides, were found in MOLE. Glycosides exert antibacterial effects primarily through mechanisms such as interfering with bacterial cell wall synthesis or inhibiting essential enzymes crucial for bacterial survival (40).

Moreover, phytosphingosine, an important component of sphingolipids, and emmotin A, a terpenoid, were detected in MOLE. Sphingolipids have been studied for their antibacterial effects, suggesting a potential role for phytosphingosine in enhancing MOLE's antimicrobial activity against bacterial pathogens (41). Emmotin A disrupts bacterial membrane integrity and interferes with intracellular signalling pathways crucial for bacterial survival, demonstrating significant antibacterial activity (42). The diverse array of bioactive compounds identified in MOLE through LC-MS underscores its potential as a source of natural antimicrobial agents. These compounds, including flavonoids, phenolics, alkaloids, glycosides, and lipids, contribute to MOLE's multifaceted bioactivity against bacterial pathogens.

It is possible that the antibacterial mechanisms of MOLE are likely multifactorial, involving disruption of bacterial cell walls, inhibition of essential bacterial enzymes, and interference with bacterial DNA and protein synthesis. Flavonoids and phenolic compounds, for instance, can form complexes with bacterial cell walls, leading to increased permeability and cell lysis (43). Alkaloids, on the other hand, can intercalate into bacterial DNA, preventing replication and transcription (44). However, it is important to note that while the general antibacterial mechanisms of the bioactive compounds are established in general, it is not yet definite how each specific compound identified in MOLE, contributes to its antibacterial activity. MOLE, with its rich phytochemical profile, presents a promising natural alternative or adjunct to conventional antibiotics, particularly against resistant strains like MRSA. The identification of bioactive compounds with potent antibacterial properties provides a foundation for the development of novel therapeutic agents derived from *M. oleifera*.

Despite the promising findings, our study has several limitations. Future studies should focus on elucidating the precise mechanisms of action of the identified bioactive compounds to optimize their use as therapeutic agents. The minimum inhibitory concentration (MIC) and time-kill kinetics were not assessed in this investigation, which could have yielded more comprehensive information regarding the bactericidal effects of MOLE. Additionally, the disc diffusion method employed in this study does not offer a comprehensive representation of MOLE's antimicrobial potential, as it predominantly measures diffusion-based inhibition and does not address bactericidal or bacteriostatic properties. To thoroughly understand the antimicrobial efficacy and mode of action of MOLE, further research is required that includes MIC, Minimum Bactericidal Concentration (MBC), and time-kill kinetics assays. Exploring the potential synergistic interactions between MOLE compounds and conventional antibiotics could enhance antibacterial efficacy and reduce the likelihood of resistance development. Additionally, conducting *in vivo* studies to assess the safety, efficacy, and pharmacokinetics of MOLE and its bioactive compounds will be essential for translating these findings into clinical applications.

CONCLUSIONS

In conclusion, MOLE demonstrates potent antimicrobial susceptibility against MRSA and MSSA. MOLE's dose-dependent efficacy against *S. aureus* strains suggests it as a viable treatment option for antibiotic-resistant bacterial strains, particularly MRSA. *E. coli* has shown to be resistant to MOLE, suggesting its antibacterial properties may be more specific to Gram-positive bacteria.

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