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Antibacterial activity of *Moringa oleifera* leaves extract against vancomycin resistant *Enterococcus faecalis* Clinical Isolates

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Abstract

Vancomycin-resistant *Enterococcus faecalis* (VRE) is a high-priority pathogen with limited treatment options. Medicinal plants offer promising alternatives due to their multi-target mechanisms. This study evaluated the antibacterial activity of *Moringa oleifera* leaf extract against VRE clinical isolates. Three Clinical isolates were phenotypically confirmed using Gram staining and biochemical tests. Vancomycin resistance was determined by disk diffusion. Ethanolic leaf extract was prepared, screened for phytochemical compounds and tested for antibacterial activity using agar well diffusion, broth microdilution (MIC), and minimum bactericidal concentration (MBC) assays. All the *E. faecalis* isolates (100%) were vancomycin-resistant (≤14 mm). Phytochemical screening of the *M. oleifera* ethanolic leaf extract revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids. The extract exhibited concentration-dependent inhibition against the VRE isolates with zones of inhibition ranging from 9–11 mm (25 mg/mL) to 20–24 mm (200 mg/mL). The MIC was 6.25 mg/mL and MBC was 12.5 mg/mL (MBC/MIC ratio = 2.0), indicating bactericidal activity. The *M. oleifera* leaf extract demonstrated significant bactericidal activity against phenotypically confirmed VRE, supporting its potential as an alternative therapeutic agent. Further studies on compound isolation, synergy, and *in vivo* efficacy are recommended.

Keywords

Antibacterial activity, antimicrobial resistance, Enterococcus faecalis, Moringa oleifera, phytochemicals.

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INTRODUCTION

Enterococcus faecalis is a Gram-positive, facultatively anaerobic, catalase-negative coccus that normally forms part of the gastrointestinal microbiota of humans and animals. Under normal conditions, it exists as a commensal organism and does not usually cause disease. However, its ability to survive under a wide range of environmental conditions allows it to become an opportunistic pathogen when host defences are weakened or the normal microbial flora is disturbed. This shift from commensalism to pathogenicity is particularly important among hospitalized and immunocompromised patients, where *E. faecalis* has been associated with infections such as urinary tract infections, wound infections, bloodstream infections, endocarditis, and intra-abdominal infections (García-Solache & Rice, 2019; Murray *et al.*, 2020; Sood *et al.*, 2020).

The clinical importance of *E. faecalis* is further increased by its ability to acquire resistance to several antimicrobial agents. Enterococci have intrinsic characteristics that make them less susceptible to many antibiotics, while exposure to antimicrobial drugs can promote the acquisition of additional resistance mechanisms. Their ability to acquire and transfer resistance genes through mobile genetic elements and horizontal gene transfer has contributed to the emergence of multidrug-resistant strains (Miller et al., 2020). As a result, enterococcal infections can be increasingly difficult to treat, particularly in healthcare settings where antimicrobial use and selection pressure are high. The emergence of strains resistant to important therapeutic agents therefore presents a significant challenge to effective infection management and control.

One of the most clinically important forms of enterococcal resistance is resistance to vancomycin. Vancomycin has long been an important treatment option for serious infections caused by Gram-positive bacteria, including enterococcal infections. However, the emergence and spread of vancomycin-resistant enterococci (VRE) have reduced the effectiveness of this treatment option. Vancomycin resistance commonly results from changes in the bacterial cell-wall precursor, which reduce the ability of the antibiotic to bind to its target and interfere with cell-wall synthesis. Resistance genes such as vanA and vanB are particularly important because they can confer high-level glycopeptide resistance and contribute to the persistence and spread of resistant strains (Ahmed & Baptiste, 2018; Miller et al., 2020).

Vancomycin-resistant *E. faecalis* is of particular concern because infections caused by these organisms may leave clinicians with limited treatment options. Drugs such as linezolid, daptomycin, and tigecycline may be used in the management of VRE infections, but their use can be limited by factors such as adverse effects, cost, pharmacological considerations, and the possible development of additional resistance (Yim et al., 2021). Dependence on a relatively small number of effective antimicrobial agents creates further concern as resistance continues to increase. This situation highlights the need to investigate alternative and complementary sources of antimicrobial agents that may help address infections caused by resistant organisms.

The increasing global burden of antimicrobial resistance has renewed interest in medicinal plants as potential sources of antimicrobial compounds (Garba et al., 2019). Plants have been used in traditional medicine for centuries, and many contain secondary metabolites with biological activities that may be useful against pathogenic microorganisms. (Ashraf et al., 2023). Plant-derived compounds may act through several mechanisms, including disruption of bacterial cell membranes, interference with enzymes, changes in cellular permeability, and inhibition of essential cellular processes. Phytochemicals such as flavonoids, alkaloids, tannins, saponins, phenolic compounds, and steroids have been associated with various antimicrobial activities (Górniak et al., 2019; Khameneh et al., 2019; Othman et al., 2019).

One medicinal plant that has attracted considerable interest is *Moringa oleifera* Lam., commonly known as the "miracle tree." *M. oleifera* is widely distributed and is valued for its nutritional, medicinal, and economic importance. Different parts of the plant, including the leaves, seeds, bark, roots, and flowers, have been investigated for their biological properties (Leone et al., 2015; Garba et al., 2021). The leaves are of particular interest because they contain a variety of bioactive compounds and are commonly consumed or used in traditional preparations. Studies have reported antioxidant, anti-inflammatory, nutritional, and antimicrobial properties associated with *M. oleifera*, making it a potential source of compounds with therapeutic value (Leone et al., 2015; Vergara-Jimenez et al., 2017).

The antimicrobial activity of *M. oleifera* has been linked, in part, to its diverse phytochemical composition. Flavonoids and other phenolic compounds may interfere with bacterial structures and metabolic processes, while alkaloids may exert antimicrobial effects through interactions with cellular components. Tannins can bind to proteins and interfere with microbial enzymes and other cellular functions, while saponins have been associated with disruption of bacterial membranes. The presence of several classes of phytochemicals may therefore contribute to the antimicrobial activity of *M. oleifera* extracts (Amabye & Tadesse, 2016; Górniak et al., 2019; Mukhtar et al., 2017). However, the activity of a plant extract can vary depending on factors such as the plant part used, geographical location, extraction solvent, concentration, and susceptibility of the target organism.

Previous studies have shown that *M. oleifera* extracts can inhibit several clinically important bacterial pathogens (Mukhtar et al., 2017; Garba et al., 2021). Antibacterial activity has been reported against organisms such as *Staphylococcus aureus*, *Bacillus subtilis*, and other pathogenic bacteria, suggesting that the plant contains compounds with activity against both Gram-positive and Gram-negative organisms (Amabye & Tadesse, 2016; Muhammad et al., 2016; Mukhtar et al., 2017). This antimicrobial potential is particularly relevant in the context of antimicrobial resistance because plant-derived compounds may provide additional sources of antimicrobial agents for further research and development. However, information on the activity of *M. oleifera* against highly resistant enterococci remains relatively limited.

Investigating medicinal plants against VRE is important because the treatment of resistant enterococcal infections requires alternatives to conventional antibiotics. Previous studies have reported antimicrobial activity of plant-derived compounds against vancomycin-resistant enterococci and other multidrug-resistant bacteria (Lee et al., 2011), suggesting that phytochemicals may have potential as alternative or complementary antimicrobial agents (Lee et al., 2011; Tanaka et al., 2004). Studies involving *M. oleifera* have also suggested that its antimicrobial activity may be enhanced when combined with conventional antibiotics, raising the possibility of future applications involving synergistic antimicrobial therapy (Mabrouk et al., 2022). However, the activity of *M. oleifera* leaf extract against phenotypically confirmed vancomycin-resistant *E. faecalis* clinical isolates remains an area that requires further investigation.

MATERIALS AND METHODS

Study Area

The study was conducted at the Federal Teaching Hospital (FTH), Gombe. The hospital is situated within Gombe metropolis, the administrative capital of the state. Gombe metropolis lies approximately between latitudes 10°N and 11°N of the Equator and is located within the Sudan Savannah ecological zone. Gombe State covers a land area of approximately 18,000 km² and had a population of about 2.5 million people according to the 2006 population census (**Figure 1**) (Oghenebrume, 2021).

Federal Teaching Hospital, Gombe, was established in 1996 to provide specialized and tertiary healthcare services to the people of Gombe State and neighbouring areas. The hospital also serves as a referral centre for patients requiring specialized medical care from surrounding communities and other healthcare facilities. It has an estimated bed capacity of about 500, with an average bed occupancy rate of approximately 90% to 95%. The high bed occupancy reflects the considerable demand for healthcare services at the facility and highlights its role as an important tertiary healthcare centre in the region.

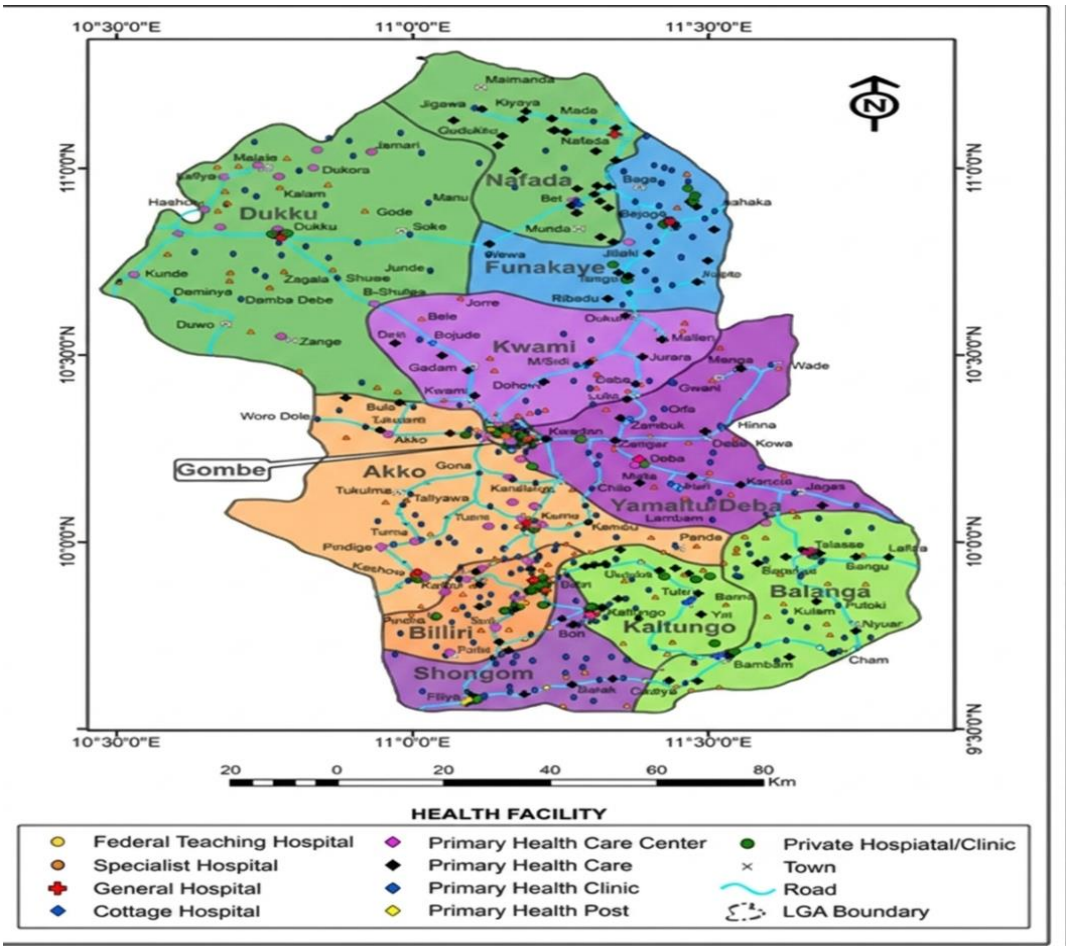


Figure 1: Map of Gombe State indicating the location of Federal Teaching Hospital, Gombe (Oghenebrume, 2021).

Source of Clinical Isolates

Four (4) clinical isolates of *Enterococcus faecalis* were collected from the Microbiology and Immunology Laboratory of the Federal Teaching Hospital, Gombe.

Confirmation of *Enterococcus faecalis*

Suspected *E. faecalis* isolates were confirmed using Gram staining, catalase test, salt tolerance (6.5% NaCl), and bile esculin hydrolysis (Murray et al., 2020).

Gram's Staining

A smear of the bacterial colony was prepared on microscopic slide and heat-fixed. Crystal Violet was applied for 1min and rinsed with distilled water. Gram's Iodine was applied for 1min and rinsed with water. Decolorization was performed using ethanol for 10-15sec and rinsed with water. Safranin was applied for 30 sec and rinsed with water. The slide was air-dried and observed the smear under oil immersion objective lens (100x) (Saleh et al., 2024).

Catalase Test

Small portion of a bacterial colony was transferred onto a clean glass slide using a sterile loop. One drop of 3% H_2O_2 was added directly onto the smear and observed immediately for bubble formation (effervescence) as suspected positive test (Murray et al., 2020).

Salt Tolerance Test

A loopful of each test organism was inoculated into 3 separate tubes of BHI broth supplemented with 6.5% NaCl. The inoculated tubes were incubated at 37°C for 24hours. Tubes were examined visually for turbidity development at 24-hour intervals (CLSI, 2023).

Bile Esculin Hydrolysis Test

A single colony of the test organism was streaked onto the surface of a bile esculin agar slant. The medium was inoculated heavily to ensure adequate growth. The inoculated medium was incubated aerobically at 37°C for 24hours. Following incubation, the medium was

examined for growth and a distinct color change (Murray et al., 2020).

Antibiotic Susceptibility Testing

The antibiotic susceptibility profile of *Enterococcus faecalis* clinical isolates to vancomycin was determined using the disk diffusion method as outlined by the Clinical and Laboratory Standards Institute (CLSI,2023). A bacterial suspension was prepared in sterile saline and adjusted to a turbidity equivalent of 0.5 McFarland standard (~1.5 × 10^8 CFU/mL). Within 15 minutes of adjustment, a sterile cotton swab was dipped into the suspension, excess fluid was removed and swabbed on Mueller-Hinton Agar (MHA) plate in three directions to ensure confluent growth (Abduljabbar et al., 2025). A 30µg vancomycin disk was aseptically placed onto the center of the inoculated agar surface and gently pressed to ensure full contact. Plates were inverted and incubated aerobically at 35 ± 2°C for 18 hours. Following incubation, the diameter of the zone of inhibition around the disk was measured to the nearest millimeter using a calibrated caliper, held over a dark, non-reflecting surface. The measured zone diameters were interpreted according to the current CLSI performance standards for *Enterococcus faecalis* (CLSI,2023).

Plant material collection and extract preparation

Preparation of plant material

The leaves were washed thoroughly with distilled water to remove dirt (Abd Rani et al., 2018) and shade-dried at room temperature (25–30°C) for 7–10 days to prevent degradation of heat-sensitive bioactive compounds (Leone et al., 2015). The dried leaves were then ground into a fine powder using an electric blender and stored in an airtight container at 4°C until further use (Vergara-Jimenez et al., 2017).

Extract Preparation:

Powdered leaves of *Moringa oleifera* (100 g) were macerated in 70% ethanol (1:10 w/v) for 72 hours at room temperature with occasional shaking. Seventy percent (70%) ethanol was selected to extract both polar and non-polar compounds (Leone et al., 2018). The mixture was filtered, and evaporated the solvent using a rotary evaporator at 40°C (Vergara-Jimenez et al., 2017).

Phytochemical Screening

Following the preparation of the *Moringa oleifera* leaf extract, a comprehensive qualitative phytochemical screening was carried out to detect the presence of diverse classes of secondary metabolites.

3.9.1 Test for Alkaloids

Alkaloid detection was carried out using Meyer's reagent. Three millilitres of the extract solution was treated with three drops of Meyer's reagent. The formation of a creamy white or pale-yellow precipitate was taken as evidence of alkaloids (Mukhtar et al., 2017).

3.9.2 Test for Tannins

The presence of tannins was assessed by adding three drops of 5% (w/v) ferric chloride solution to 2 mL of the extract solution previously diluted with 2 mL of distilled water. The development of a green-black or blue-black precipitate confirmed the presence of tannins (Mukhtar et al., 2017).

3.9.3 Test for Steroids

Steroid detection was performed using the Salkowski test. One millilitre of the extract solution was combined with 1 mL of chloroform. Subsequently, 1 mL of concentrated sulfuric acid was carefully layered along the inner wall of the test tube. A red coloration in the chloroform layer or a greenish-blue colour at the interface indicated the presence of steroids (Mukhtar et al., 2017).

3.9.4 Test for Flavonoids

Flavonoid content was determined by adding 1 mL of 10% (w/v) sodium hydroxide solution to 1 mL of the extract solution diluted in 10 mL of distilled water. The appearance of an intense yellow colour that became colourless upon the addition of a few drops of dilute hydrochloric acid was considered positive for flavonoids (Amabye & Tadesse, 2016).

3.9.5 Test for Saponin

Saponin detection was carried out using the froth test. Two millilitres of the extract solution was placed in a stoppered test tube containing 5 mL of distilled water. The tube was shaken vigorously for 30 seconds. The

persistence of a stable froth layer of at least 1 cm for 10 minutes was regarded as evidence of saponins (Mukhtar et al., 2017).

Antibacterial Assay

The antibacterial activity of the *M. oleifera* leaf extract was evaluated using the agar well diffusion method. Wells measuring 6 mm in diameter were made in Mueller-Hinton agar plates previously inoculated with a bacterial suspension adjusted to 0.5 McFarland standard. Different concentrations of the extract (200, 100, 50, and 25 mg/mL) were added to the wells at a volume of 100 µL per well. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimetres (Muhammad et al., 2016; Garba et al., 2021).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) of the extract was determined using the broth microdilution method with two-fold serial dilutions ranging from 200 mg/mL to 1.56 mg/mL, following CLSI (2023) guidelines. The MIC was recorded as the lowest concentration of the extract that showed no visible bacterial growth. To determine the minimum bactericidal concentration (MBC), samples from wells showing no visible growth were sub-cultured onto fresh agar plates and incubated. The MBC was defined as the lowest concentration that produced no bacterial growth on the subculture (French, 2006).

RESULTS

Identification of Clinical Isolates

Three of the four isolates (75%) were identified as *E. faecalis*, while one isolate (25%) was identified as *Staphylococcus spp.* All the three *E. faecalis* isolates were catalase-negative, capable of growing in 6.5% NaCl, and able to hydrolyze esculin in the presence of bile **(Table 1).**

Table 1: Biochemical characteristics of the Clinical Isolates

S/N	ID	ISO	GR	Cat	ST	BEH	ORG
1.		ISO 01	+	+	+	-	*Staphylococcus Spp.*
2.		ISO 02	+	-	+	+	*Enterococcus faecalis*
3.		ISO 03	+	-	+	+	*Enterococcus faecalis*
4.		ISO 04	+	-	+	+	*Enterococcus faecalis*

Key: GR = Gram reaction, Cat = Catalase, ST = Salt tolerance, BEH = Bile esculin hydrolysis; +=Positive, - =Negative

Antibiotic Susceptibility Profile

As presented in Table 2, all the three (3) clinical isolates of *Enterococcus faecalis* were resistant to vancomycin indicating complete resistance among the isolates tested.

Table 2. Antibiotic Susceptibility Profiles of *E. faecalis* Isolate.

S/N	ISO ID	Antibiotic	R	S	I
1.	ISO 02	Vancomycin	+	-	-
2.	ISO 03	Vancomycin	+	-	-
3.	ISO 04	Vancomycin	+	-	-

KEY: + = Positive, - = Negative, R= Resistance, S= Susceptible, I = Intermediate, ISO ID = Isolate identification number

Phytochemical Screening

The phytochemical screening of the extract revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids (Table 3).

Table 3: Phytochemical constituents of *Moringa oleifera* leaf extract

S/N	Phytochemical	Inference
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tannin	+
4.	Saponin	+
5.	Steroids	+

KEY: + = Positive (presence of phytochemical constituent), – = Negative (absence of phytochemical constituent)

Antibacterial Activity of *M. oleifera* extract

The extract demonstrated concentration-dependent antibacterial activity against the VRE isolates, with the zones of inhibition increasing from 9–11 mm at 25 mg/mL to 20–24 mm at 200 mg/mL (Table 4). This indicates that higher concentrations of the extract produced greater inhibition of bacterial growth. A representative plate demonstrating the zone diameter produced by the extract against the test isolates is displayed in Figure 2.

Table 4: Antibacterial Activity of *Moringa oleifera* leaf Extract Against VRE Isolates

S/N	Isolate code	Zone diameter (mm) per concentration (mg/ml)			
		200	100	50	25
1.	VRE 02	23	20	18	10
2.	VRE 03	22	19	15	10
4.	VRE 04	23	19	17	11

Key: VRE= Vancomycin resistant *Enterococcus faecalis*.

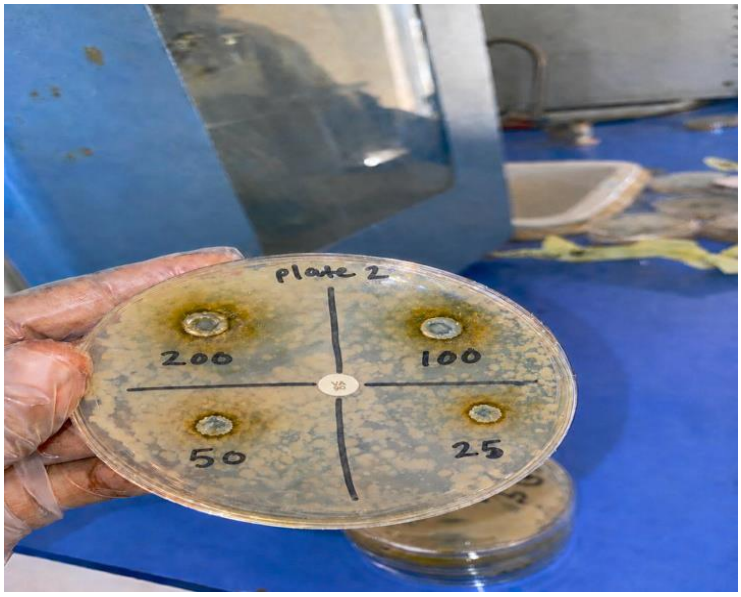


Figure 2: Zone of inhibition of the extract against VRE on Mueller Hinton agar plate

Minimum Inhibitory and Bactericidal Concentrations

The minimum inhibitory concentration (MIC) of the extract was 6.25 mg/mL(Table 5), while the minimum bactericidal concentration (MBC) was 12.5 mg/mL (Table 6). The resulting MBC/MIC ratio of 2.0 indicates that the extract exhibited bactericidal activity against the VRE isolates.

Table 5: Minimum Inhibitory Concentration (MIC) of *Moringa oleifera* Ethanolic Extract Against Vancomycin resistant *enterococcus faecalis*

S/N	Concentrations mg/mL	Result
1.	200	-
2.	100	-
3.	50	-
4.	25	-
5.	12.5	-
6.	6.25	-
7.	3.125	+
8.	1.56	+

KEY: + = Turbid (visible bacterial growth), - = Non-turbid (absence of bacterial growth)

Table 6: Minimum Bactericidal Concentration (MBC) of *Moringa oleifera* Extract Against VRE

S/N	Concentrations (mg/ml)	Subculture result
1.	200	-
2.	100	-
3.	50	-
4.	25	-
5.	12.5	-
6.	6.25	+

KEY: - = No growth on subculture (bactericidal effect), + = Growth on subculture (bacteriostatic effect only

DISCUSSION

The present study investigated the antibacterial activity of ethanolic extract of *Moringa oleifera* leaves against vancomycin-resistant *Enterococcus faecalis* clinical isolates. The phenotypic identification aligned with the established biochemical characteristics of the test organisms. The isolates were Gram-positive cocci, catalase-negative, ability to grow in 6.5% NaCl, and positive for bile esculin hydrolysis. These characteristics are consistent with the established features of enterococci (Murray et al., 2020; Facklam & Elliott, 1995).

A major finding of the study was the complete resistance of the tested isolates to vancomycin. This finding is clinically important because vancomycin has traditionally been an important treatment option for serious infections caused by Gram-positive organisms, including enterococci. The emergence of vancomycin-resistant enterococci has reduced the usefulness of this antibiotic and created additional challenges in the treatment of enterococcal infections (Ahmed & Baptiste, 2018; Miller et al., 2020). The complete resistance observed in this study also highlights the need for continued monitoring of antimicrobial susceptibility patterns in healthcare facilities.

The presence of vancomycin-resistant *E. faecalis* is particularly concerning because resistance to vancomycin may occur together with resistance to other antimicrobial agents. Enterococci have intrinsic resistance characteristics and can acquire additional resistance determinants through genetic exchange and other mechanisms. As a result, infections caused by resistant enterococci may become increasingly difficult to treat, particularly when resistance involves several antimicrobial classes (Miller et al., 2020; Sood et al., 2020).

Phytochemical screening of the *M. oleifera* leaf extract revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids. This finding is consistent with previous reports describing the diverse phytochemical composition of *M. oleifera* leaves (Amabye & Tadesse, 2016; Mukhtar et al., 2017). The presence of several classes of secondary metabolites may partly explain the antibacterial activity observed in this study. Plant-derived phytochemicals can affect bacterial cells through different mechanisms, including disruption of cell membrane integrity, interference with enzymes and cellular proteins, changes in membrane permeability, and inhibition of essential metabolic processes (Górniak et al., 2019; Khameneh et al., 2019; Othman et al., 2019).

Flavonoids, for instance, have been widely studied for their antimicrobial properties and may interfere with bacterial cell structures and proteins. Alkaloids have also been associated with antimicrobial effects through their interaction with cellular components, while tannins may bind to proteins and interfere with microbial enzymes. Saponins, in particular, have been associated with effects on bacterial membranes. The combined presence of these phytochemicals may contribute to the antibacterial activity of the extract rather than the activity being due to a single compound (Górniak et al., 2019; Othman et al., 2019). However, because the phytochemical screening in this study was

qualitative, it was not possible to determine which specific compounds were responsible for the observed antibacterial activity.

The antibacterial assay showed a clear concentration-dependent response. At 25 mg/mL, the extract produced inhibition zones ranging from 9 to 11 mm, which increased to 15–18 mm at 50 mg/mL. At 100 mg/mL, inhibition zones ranged from 18 to 20 mm, while the highest concentration tested, 200 mg/mL, produced zones ranging from 20 to 24 mm. The progressive increase in inhibition with increasing extract concentration suggests that higher concentrations provided greater amounts of active antibacterial constituents capable of interacting with the bacterial cells. Similar antibacterial activity has been reported for *M. oleifera* leaf extracts against different pathogenic bacteria, supporting the antimicrobial potential of the plant (Amabye & Tadesse, 2016; Muhammad et al., 2016; Mukhtar et al., 2017).

The concentration-dependent response observed in this study is biologically reasonable because crude plant extracts contain mixtures of active compounds whose effects depend on their concentration. At lower concentrations, the active compounds available to interact with bacterial cells may be insufficient to produce extensive inhibition, whereas increasing the concentration provides more of these compounds, resulting in increased interference with bacterial structures and metabolic processes. The gradual increase in inhibition zones observed across the concentrations tested therefore suggests a concentration-related antibacterial effect, consistent with previous studies that have reported dose-dependent antibacterial activity for *Moringa oleifera* extracts against various bacterial pathogens. For instance, Jahan et al. (2022) reported dose-dependent inhibitory effects of *M. oleifera* leaf ethanolic extract against *Staphylococcus aureus* and *Escherichia coli* using disc diffusion, with maximum zones of inhibition observed at the highest concentration tested. Similarly, Kanu et al. (2025) documented that the highest zone of inhibition was achieved at 200 mg/mL concentration of *M. oleifera* ethanolic leaf extract against *Staphylococcus aureus*, with no susceptibility observed at lower concentrations for some organisms. These findings align with the present study's observation of concentration-dependent antibacterial activity against vancomycin-resistant *Enterococcus faecalis*.

The MIC result provided further evidence of the antibacterial effect of the extract. The MIC was 6.25 mg/mL, indicating that this was the lowest concentration at which visible bacterial growth was inhibited under the conditions of the broth microdilution assay. MIC determination provides a quantitative measure of the concentration required to inhibit bacterial growth and complements the inhibition-zone results obtained through agar well diffusion. The ability of the extract to inhibit the isolates at this concentration provides additional evidence that its antibacterial constituents remained active under broth dilution conditions.

The MBC of 12.5 mg/mL further indicated that the extract was capable of killing the bacteria rather than simply inhibiting their growth. The MBC/MIC ratio of 2.0 supports the classification of the extract as bactericidal according to the criterion applied in this study (French, 2006). The distinction between bacteriostatic and bactericidal activity is important when evaluating potential antimicrobial agents because the absence of visible growth does not necessarily mean that the organisms have been killed. The combined MIC and MBC findings therefore provide stronger evidence of antibacterial activity than the agar diffusion results alone.

The bactericidal effect observed may be related to the combined action of the phytochemicals detected in the extract. A crude plant extract contains several compounds that may act on different bacterial targets. Some may affect membrane integrity, while others may interfere with enzymes, proteins, or intracellular processes (Amabye and Tadesse, 2016). This combined activity may partly explain why the extract was effective against isolates that were resistant to vancomycin. However, the findings should

not be interpreted as evidence that the extract reverses vancomycin resistance. Rather, the results suggest that the extract contains antibacterial constituents that may act through mechanisms different from those of vancomycin.

The findings are comparable with previous studies investigating plant-derived antimicrobial agents against resistant enterococci. Tanaka et al. (2004) reported antibacterial activity of phytochemicals isolated from *Erythrina zeyheri* against vancomycin-resistant enterococci, demonstrating the potential of plant-derived compounds against organisms resistant to conventional antibiotics. Similarly, Lee et al. (2011) reported antimicrobial activity of *Alpinia officinarum* extracts against VRE and other pathogenic microorganisms. More recently, Romyasamit et al. (2024) reported antibacterial and antibiofilm activity of *Gymnema inodorum* leaf extract against vancomycin-resistant *Enterococcus faecium*. These findings, together with the results of the present study, support continued investigation of medicinal plants as potential sources of antimicrobial compounds against resistant enterococci.

The antibacterial activity observed in this study agrees with previous research on *M. oleifera*. For instance, Amabye and Tadesse (2016) reported that ethanolic extracts of *M. oleifera* leaf exhibited antibacterial activity against clinical isolates including *Staphylococcus aureus* and *Streptococcus pyogenes*, attributing the effects to flavonoids and phenolic compounds. Muhammad et al. (2016) demonstrated that ethanolic and aqueous extracts of *M. oleifera* leaf produced inhibition zones ranging from 10–18 mm against *S. aureus*, with MIC values of 6.25–12.5 mg/mL. Similarly, Mukhtar et al. (2017) reported that *Moringa* extracts exhibited antimicrobial activity against vancomycin-resistant cocci isolated from clinical specimens. The present study extends these findings by demonstrating that ethanolic *M. oleifera* leaf extract produced zones of inhibition ranging from 9–24 mm against phenotypically confirmed vancomycin-resistant *E. faecalis* clinical isolates, with an MIC of 6.25 mg/mL and MBC of 12.5 mg/mL, confirming bactericidal activity. This consistency with previous studies supports the potential of *M. oleifera* as a source of antibacterial compounds effective against resistant Gram-positive pathogens.

The findings also provide a basis for future studies investigating combinations of *M. oleifera* extract with conventional antibiotics. Previous research has suggested that *M. oleifera* may have synergistic effects when combined with selected antibiotics, although such interactions depend on the organism, antibiotic, extract composition, and experimental conditions (Mabrouk et al., 2022). Studies involving other plant-derived compounds have similarly suggested that phytochemicals may enhance the activity of conventional antimicrobial agents against resistant bacteria (Tanaka et al., 2004). Further investigation could therefore determine whether *M. oleifera* extract has synergistic, additive, or antagonistic effects when combined with antibiotics commonly used against enterococcal infections.

CONCLUSION

The ethanolic leaf extract of *Moringa oleifera* demonstrated significant concentration-dependent antibacterial activity against phenotypically confirmed vancomycin-resistant *Enterococcus faecalis* clinical isolates. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, saponins, and steroids, which likely contributed to the observed activity through multiple mechanisms. The MIC of 6.25 mg/mL and MBC of 12.5 mg/mL, with an MBC/MIC ratio of 2.0, confirm that the extract is bactericidal rather than merely bacteriostatic against VRE. These findings support the potential of *M. oleifera* leaf extract as an alternative or adjunctive therapeutic agent against vancomycin-resistant *Enterococcus faecalis*, particularly in resource-limited settings. However, further studies involving compound isolation, synergy with conventional antibiotics, and in vivo efficacy are recommended to fully establish its therapeutic potential.

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