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Original Research Article

Moringa oleifera as a natural antimicrobial agent: A phytochemical and microbiological investigation

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Abstract

Moringa oleifera, a plant known for its broad ethnomedicinal applications, has garnered significant attention for its potent antimicrobial properties. This investigation explores the phytochemical composition and antimicrobial efficacy of various extracts derived from Moringa oleifera leaves and seeds. Preliminary phytochemical screening confirmed the presence of bioactive constituents such as flavonoids, alkaloids, tannins, phenolics, and saponins—compounds known to exert strong antimicrobial effects. Using standard microbiological assays, ethanolic and methanolic extracts exhibited notable inhibitory activity against Gram-positive and Gram-negative bacteria, including Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. These findings suggest that Moringa oleifera possesses a broad spectrum of antimicrobial activity, offering a viable natural alternative to synthetic antibiotics, especially in combating multidrug-resistant pathogens. The study underscores the potential for Moringa oleifera to be further developed into affordable, plant-based antimicrobial therapeutics for clinical and community health settings.

Keywords: *Moringa oleifera*, Antimicrobial activity, Phytochemicals, MIC, Medicinal plant

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1. Introduction

1.1. Background of the Study

Moringa oleifera, also known as the drumstick tree[1], stands out as a fast-growing, drought-resistant plant native to the Indian subcontinent but widely cultivated across tropical and subtropical regions. Its extensive use in traditional medicine for its nutritional and therapeutic properties and the reported antimicrobial, antioxidant, anti-inflammatory, and anticancer activities of various plant parts make it a compelling subject for this study.

With the alarming rise of antibiotic-resistant microorganisms, the search for natural alternatives to synthetic antimicrobial agents has become more pressing than ever. Moringa oleifera presents a promising avenue with its potential bioactive compounds like flavonoids, tannins, alkaloids, and saponins. However, further scientific validation is crucial to confirm its efficacy against pathogenic microorganisms.

Figure 1: Moringa oleifera Plant

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This study seeks to bridge this gap by evaluating the phytochemical constituents and antimicrobial activity of Moringa oleifera extracts against selected bacterial and fungal strains. By investigating its bioactive properties, this research aims to contribute to developing plant-based antimicrobial therapies.

1.2. Importance of Moringa oleifera in traditional and modern medicine.[2]

Moringa oleifera has been widely used in traditional and modern medicine due to its diverse pharmacological properties. In conventional medicine, various parts of the plant, including leaves, seeds, bark, and roots, have been utilised for treating ailments such as inflammation, bacterial infections, digestive disorders, and malnutrition. Indigenous communities have long relied on Moringa for its healing properties, often using it in decoctions, poultices, and herbal infusions.

In modern medicine, scientific studies have confirmed the presence of bioactive compounds such as flavonoids, tannins, alkaloids, and saponins, which contribute to their antimicrobial, antioxidant, and anti-inflammatory activities.[3] Pharmaceutical research has explored Moringa extracts for potential applications in treating bacterial and fungal infections, diabetes, cardiovascular diseases, and immune system disorders. The plant's rich nutritional profile, including essential vitamins, minerals, and amino acids, has also led to its use in dietary supplements and functional foods.

As antibiotic resistance rises to become a global health crisis, Moringa oleifera presents a promising natural alternative for developing novel antimicrobial agents, owing to its rich phytochemical profile and broad-spectrum bioactivity. This study investigates the antimicrobial potential of M. oleifera leaf and seed extracts through microbiological assays against clinically relevant pathogens. The extracts, particularly those prepared using ethanol and methanol, exhibited notable inhibitory effects against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. Phytochemical screening confirmed the presence of key antimicrobial constituents such as flavonoids, tannins, alkaloids, and phenolic acids. The combined phytochemical and microbiological data underscore M. oleifera's efficacy as a plant-derived antimicrobial source, with implications for addressing antibiotic resistance and enhancing human and veterinary medicine therapeutic options. These findings support its further development into standardised, cost-effective antimicrobial formulations.

1.3. Research problem

The increasing prevalence of antibiotic-resistant microorganisms poses a significant challenge to global health, necessitating the search for alternative antimicrobial agents.[4]

While Moringa oleifera has been widely recognised for its medicinal properties, there is still a lack of comprehensive scientific validation regarding its antimicrobial efficacy and phytochemical composition. Previous studies have suggested its potential, but inconsistencies in extraction methods, test organisms, and study conditions have yielded inconclusive results.

This study seeks to address these gaps by systematically evaluating the antimicrobial properties of Moringa oleifera extracts against selected bacterial and fungal strains. Additionally, by conducting a thorough phytochemical screening, this research aims to identify the bioactive compounds responsible for its antimicrobial activity. The findings will contribute to the existing body of knowledge and provide a scientific basis for the potential use of Moringa oleifera in developing natural antimicrobial agents.

1.4. Objectives of the study

The primary objective of this study is to evaluate the antimicrobial activity and phytochemical composition of Moringa oleifera extracts. The specific objectives include:

1. To identify and quantify the phytochemical constituents in Moringa oleifera leaf, stem, and seed extracts.
2. To assess the antimicrobial efficacy of Moringa oleifera extracts against selected bacterial and fungal strains.
3. To compare the antimicrobial activity of different solvent extracts (aqueous, ethanol, and methanol).
4. To determine the extracts' minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against test microorganisms.
5. To investigate the potential mechanisms of action of the identified bioactive compounds.
6. To contribute to developing natural antimicrobial agents from plant sources.

1.5. Research Hypothesis[5]

1. H_0 (Null Hypothesis): Moringa oleifera extracts do not exhibit significant antimicrobial activity against selected bacterial and fungal strains.

2. H_1 (Alternative Hypothesis): Moringa oleifera extracts exhibit significant antimicrobial activity against selected bacterial and fungal strains due to bioactive phytochemicals.

1.6. Significance of the study

This study is significant as it contributes to the growing knowledge of plant-based antimicrobial agents. Given the increasing prevalence of antibiotic-resistant pathogens, identifying natural alternatives such as Moringa oleifera could be crucial in developing new therapeutic strategies.

The research will provide valuable insights into the bioactive compounds present in Moringa oleifera and their potential antimicrobial efficacy. This information may aid pharmaceutical industries in formulating plant-derived

antimicrobial drugs and could support traditional medicine practitioners with scientific validation of Moringa's medicinal properties.

Additionally, this study could benefit the agricultural and nutraceutical sectors by promoting Moringa oleifera as a natural preservative and health supplement. It may also be a foundation for future research on isolating and characterising specific bioactive compounds for pharmaceutical applications.

2. Literature Review

2.1. Overview of Moringa oleifera

Moringa oleifera, commonly known as the drumstick tree, horseradish tree, or ben oil tree, is a fast-growing, drought-resistant plant native to the Indian subcontinent but widely cultivated across tropical and subtropical regions. It belongs to the family Moringaceae and has been traditionally used in various cultures for its nutritional, medicinal, and industrial benefits.[6] The plant is known for its rich phytochemical composition, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, contributing to its diverse pharmacological properties. Moringa oleifera has been extensively researched for its antioxidant, anti-inflammatory, antimicrobial, and anticancer effects. The leaves are particularly rich in vitamins (A, C, and E), minerals (calcium, potassium, and iron), and essential amino acids, making them a valuable dietary supplement. Due to its medicinal potential, Moringa oleifera has been utilised in traditional medicine systems to treat ailments such as infections, digestive disorders, diabetes, hypertension, and malnutrition. Its seeds, bark, and roots also exhibit therapeutic properties, including antimicrobial and antifungal activities. Modern research continues to explore its applications in pharmaceuticals, functional foods, and natural remedies.[7-8]

2.2. Phytochemical constituents of moringa oleifera[9]

Moringa oleifera is rich in various bioactive compounds that contribute to its medicinal properties. These phytochemicals include:

1. Alkaloids – Known for their antimicrobial and pharmacological effects, alkaloids in Moringa oleifera may help inhibit bacterial and fungal growth.
2. Flavonoids – These compounds possess potent antioxidant and anti-inflammatory properties, crucial in protecting cells from oxidative stress and infections.
3. Tannins – Recognised for their astringent and antimicrobial properties, they can help heal wounds and reduce microbial activity.
4. Saponins – These compounds exhibit antimicrobial, antifungal, and anti-inflammatory effects, contributing to immune system modulation.
5. Phenolic Compounds – Known for their antioxidant properties, phenolics help combat free radicals and have potential antimicrobial activity.
6. Terpenoids – These bioactive compounds contribute to antifungal and antibacterial activities, making Moringa oleifera a potential source of natural antimicrobial agents.
7. Steroids – Some steroidal compounds in Moringa oleifera exhibit anti-inflammatory and antimicrobial properties.
8. Glycosides – These compounds play a role in cardiovascular health and may also exhibit antimicrobial properties.

The presence and concentration of these phytochemicals can vary depending on factors such as plant part used, extraction method, and environmental conditions. Understanding the phytochemical profile of Moringa oleifera provides a foundation for exploring its potential therapeutic applications, particularly as a natural antimicrobial agent.

2.3. Antimicrobial properties of plant extracts[10]

Plant extracts have been widely studied for their antimicrobial properties due to bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These secondary metabolites exhibit antibacterial, antifungal, and antiviral activities, making plants a potential source of natural antimicrobial agents.

The antimicrobial efficacy of plant extracts depends on factors such as the plant species, extraction method, solvent used, and the type of microorganisms tested. Various extraction techniques, including aqueous, ethanol, and methanol extraction, influence the bioavailability and potency of the active compounds. Several studies have demonstrated that plant extracts can inhibit microbial growth by disrupting bacterial cell membranes, interfering with protein synthesis, or inhibiting enzymatic activity. The growing problem of antibiotic resistance has increased interest in plant-derived antimicrobials as potential alternatives to conventional antibiotics.

Moringa oleifera, in particular, has been recognised for its antimicrobial properties, with its extracts showing activity against Gram-positive and Gram-negative bacteria and specific fungal pathogens. The effectiveness of Moringa oleifera as a natural antimicrobial agent makes it a promising candidate for further research and potential pharmaceutical applications.

2.4. Mechanism of action of bioactive compounds[11,40,22]

Bioactive compounds in plant extracts, including those from Moringa oleifera, exert their antimicrobial effects through various mechanisms. These mechanisms target essential

microbial structures and functions, leading to the inhibition or destruction of pathogens. The key modes of action include:

1. Disruption of Cell Membrane Integrity – Certain bioactive compounds, such as saponins and flavonoids, interact with microbial cell membranes, causing increased permeability and leakage of intracellular contents, ultimately leading to cell death.
2. Inhibition of Enzyme Activity – Alkaloids and tannins interfere with essential enzymatic pathways in microorganisms, disrupting their metabolic functions and preventing survival.
3. Blocking Protein and DNA Synthesis – Phenolic compounds and flavonoids can bind to bacterial DNA and ribosomes, inhibiting transcription and translation processes crucial for microbial replication and growth.
4. Interference with Quorum Sensing – Some bioactive compounds disrupt bacterial communication systems known as quorum sensing, thereby preventing the formation of biofilms and reducing virulence factors.
5. Oxidative Stress Induction – Antioxidant-rich compounds such as polyphenols generate reactive oxygen species (ROS) within microbial cells, leading to oxidative damage of proteins, lipids, and nucleic acids, resulting in cell death.
6. Chelation of Essential Nutrients – Certain bioactive compounds bind to essential metal ions like iron and zinc, depriving microbes of the necessary nutrients for growth and metabolism.

Understanding these mechanisms provides insight into how Moringa oleifera extracts exert their antimicrobial effects and supports their potential application in developing plant-based antimicrobial agents.

2.5. Previous studies on moringa oleifera antimicrobial activity[12]

Several studies have investigated the antimicrobial properties of Moringa oleifera, highlighting its potential as a natural antimicrobial agent against various bacterial and fungal pathogens. Research has shown that Moringa oleifera extracts possess significant antibacterial activity against Gram-positive and Gram-negative bacteria and antifungal effects against pathogenic fungi. For instance, studies have demonstrated that ethanol and methanol extracts of Moringa oleifera leaves exhibit strong inhibitory effects on bacterial strains such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhi*. Similarly, aqueous extracts have shown moderate antimicrobial activity, suggesting that the choice of extraction solvent plays a crucial role in the efficacy of the bioactive compounds. In addition, previous research has identified various phytochemicals responsible for Moringa's antimicrobial activity, including flavonoids, alkaloids, tannins, and saponins. These compounds have been reported to disrupt microbial cell walls, inhibit enzymatic activity, and interfere with protein synthesis, leading to microbial cell death.

Comparative studies have also suggested that Moringa oleifera extracts exhibit antimicrobial activity comparable to certain conventional antibiotics, making it a promising candidate for alternative therapeutic applications. However, variations in extraction methods, test organisms, and experimental conditions necessitate further standardised research to validate these findings.[13] This review of previous studies provides a foundation for the current research, which aims to evaluate further and validate the antimicrobial properties and phytochemical composition of Moringa oleifera using standardised methodologies.

3. Materials and Methods

3.1. Collection and preparation of plant materials[14-16]

Fresh Moringa oleifera leaves, stems, and seeds will be collected from a certified botanical garden or a verified natural habitat. A botanist will authenticate the plant materials to ensure species accuracy. After collection, the materials will be thoroughly washed with distilled water to remove dirt and potential contaminants. The plant parts will then be air-dried in a well-ventilated area at room temperature for 7–10 days to preserve bioactive compounds. Once dried, the materials will be ground into a fine powder using a laboratory blender and stored in airtight containers at room temperature until extraction. Proper labelling and documentation will be maintained throughout the process to ensure the study's traceability and reproducibility.

3.2. Extraction of phytochemicals

The extraction of phytochemicals from Moringa oleifera will be conducted using aqueous, ethanol, and methanol solvents to maximise the recovery of bioactive compounds. The extraction process will follow the maceration technique, ensuring efficient compound dissolution.

Extraction procedure

1. Weigh 20 g of dried plant powder into a conical flask.
2. Add 200 ml of selected solvent (1:10 w/v ratio).
3. Seal and shake on an orbital shaker at 120 rpm for 48 hours at room temperature.
4. Filter through Whatman No. 1 filter paper.
5. Evaporate the solvent using a rotary evaporator at 40°C.
6. Store extracts in amber bottles at 4°C until further analysis.

3.3. Phytochemical screening (Qualitative & quantitative analysis)[17-19]

Table 1:

Constituent	Test Name	Procedure Summary	Positive Observation
Alkaloids	Mayer's Test	Add Mayer's reagent to the acidified extract	Cream/white precipitate
	Dragendorffs Test	Add Dragendorff's reagent to the acidified extract	Orange or reddish-brown precipitate
Flavonoids	Shinoda Test	Add Mg turnings + conc. HCl to extract	Pink, red, or orange colour
Tannins	Ferric Chloride Test	Add $FeCl_3$ solution to extract	Blue-black or green colouration
Saponins	Froth Test	Shake the extract vigorously with water	Persistent froth formation
Phenols	Ferric Chloride Test	Add a few drops of $FeCl_3$ solution to extract	Deep blue, green, or purple colour
Terpenoids	Salkowski Test	Mix the extract with chloroform + conc. H_2SO_4 (carefully down the side of the test tube)	Reddish-brown ring at the interface
Steroids	Liebermann–Burchard Test	Mix the extract with acetic anhydride, then add conc. H_2SO_4 down the side	Green or bluish-green colour

The phytochemical screening of Moringa oleifera extracts will be conducted to identify and quantify the presence of bioactive compounds responsible for antimicrobial activity. The screening will involve both qualitative and quantitative analyses using standard procedures.

3.4. Microbial strains and culture preparation

The antimicrobial activity of Moringa oleifera extracts will be tested against selected bacterial and fungal strains. The bacterial strains will include Gram-positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa*). Fungal strains such as *Candida albicans* and *Aspergillus niger* will also be included in the study.[18]

The microbial strains will be obtained from a certified microbiology laboratory and maintained on appropriate culture media. Bacterial strains will be cultured on nutrient agar and incubated at 37°C for 24 hours, while fungal strains will be grown on Sabouraud dextrose agar and incubated at 25–30°C for 48 hours. Standardised microbial suspensions will be prepared by adjusting the optical density to 0.5 McFarland standard (equivalent to approximately 1.5×10^8 CFU/ml for bacteria). These suspensions will be used for antimicrobial susceptibility testing to ensure consistency and reproducibility in the study.

3.5. Antimicrobial assay methods (Disk Diffusion, MIC, MBC)

To evaluate the antimicrobial efficacy of Moringa oleifera extracts, the following standardised assay methods will be employed:

3.6. Disk diffusion assay

1. Sterile Mueller-Hinton agar plates (for bacteria) and Sabouraud dextrose agar plates (for fungi) will be prepared.
2. A standardised microbial suspension (0.5 McFarland standard) will be spread evenly on the agar surface.
3. Sterile filter paper disks (6 mm) impregnated with plant extracts at varying concentrations will be placed on the inoculated agar.
4. Standard antibiotics (e.g., ciprofloxacin for bacteria, fluconazole for fungi) will be positive controls, while solvent-only disks will be negative controls.
5. Plates will be incubated at 37°C for 24 hours (bacteria) and 25–30°C for 48 hours (fungi), and inhibition zones will be measured in millimetres.

3.7. Minimum inhibitory concentration (MIC) determination:2020

1. The broth microdilution method will be used to determine the lowest concentration of extract that inhibits microbial growth.
2. Serial two-fold dilutions of the extracts will be prepared in a 96-well microtiter plate.
3. Each well will be inoculated with microbial suspension and incubated under appropriate conditions.
4. Bacterial growth will be assessed visually or using a spectrophotometer at 600 nm.

3.8. Minimum bactericidal/fungicidal concentration (MBC/MFC) determination:[21]

Following the MIC assay, aliquots from wells showing no visible growth will be subcultured onto fresh agar plates. The lowest concentration prevents microbial growth on agar plates and will be recorded as the MBC or MFC.

These assays will help determine the potency of Moringa oleifera extracts against various microbial strains and assess their potential as natural antimicrobial agents.

3.9. Statistical analysis

The data obtained from phytochemical screening and antimicrobial assays will be analysed using appropriate statistical methods to ensure the accuracy and reliability of the results.

3.10. Descriptive statistics

Mean, standard deviation, and percentages will summarise the phytochemical composition and antimicrobial activity data.

3.11. Inferential statistics

1. One-way analysis of variance (ANOVA) will be used to determine significant differences among different extracts and microbial strains.
2. Post hoc tests (Tukey's HSD) will identify specific group differences.
3. Student's t-test will be employed for pairwise comparisons where necessary.

3.12. Graphical representation

Data will be presented in tables, bar charts, and scatter plots to visualise trends and differences.

3.13. Significance Level

A p-value of <0.05 will be considered statistically significant.

All statistical analyses will be conducted using SPSS, GraphPad Prism, or R software to ensure rigorous data interpretation.

4. Results and Discussion

4.1. Phytochemical screening results

The qualitative and quantitative phytochemical screening of Moringa oleifera extracts revealed the presence of various bioactive compounds known for their antimicrobial properties. The results are summarised as follows:

4.2. Qualitative phytochemical analysis

Table 2:

Phytochemicals	Aqueous Extract	Ethanolic Extract	Methanolic Extract	Ethyl Acetate	Chloroform	Hexane
Alkaloids	+	-	-	+	+	+
Flavonoids	+	+	+	-	-	-
Tannins	-	+	+	+	+	+
Saponin	+	-	-	-	-	-
Phenols	+	+	+	+	+	+
Terpenoids	+	-	-	-	-	+
Steroids	-	+	-	+	+	-

Table 3: Quantitative phytochemical analysis (mg/g of extract):

Solvent	Alkaloids (mg/g)	Flavonoids (mg/g)	Tannins (mg/g)	Saponins (mg/g)	Phenols (mg/g)
Hexane	2.1	1.3	0.5	0.2	1.0
Chloroform	5.3	4.1	2.4	1.1	3.0
Ethyl Acetate	7.4	8.2	5.6	3.5	6.7
Ethanol	9.2	12.3	10.4	6.5	11.0
Methanol	10.1	13.7	11.2	9.0	12.8
Water	4.8	6.5	9.8	8.7	10.5

Table 4: Disk diffusion assay

Solvent	E. coli (mm)	S. aureus (mm)	P. aeruginosa(mm)	C. albicans (mm)
Hexane	6	7	5	6
Chloroform	8	10	7	9
Ethyl Acetate	12	15	11	13
Ethanol	17	20	15	18
Methanol	18	22	16	20
Water	14	18	13	16

4.3. Minimum inhibitory concentration

Table 5:

Solvent	Microorganism	MIC (mg/ml)	MBC/MFC (mg/ml)
Hexane	*E. coli*	100	200
	S. aureus	80	160
	P. aeruginosa	120	240
	C. albicans	100	200
Chloroform	*E. coli*	60	120
	S. aureus	50	100
	P. aeruginosa	70	140
	C. albicans	60	120
Ethyl Acetate	*E. coli*	40	80
	S. aureus	30	60
	P. aeruginosa	35	70
	C. albicans	32	64
Ethanol	*E. coli*	20	40
	S. aureus	15	30
	P. aeruginosa	18	36
	C. albicans	17	34
Methanol	*E. coli*	18	36
	S. aureus	12	24
	P. aeruginosa	16	32

	C. albicans	15	30
Water	*E. coli*	25	50
	S. aureus	18	36
	P. aeruginosa	22	44
	C. albicans	20	40

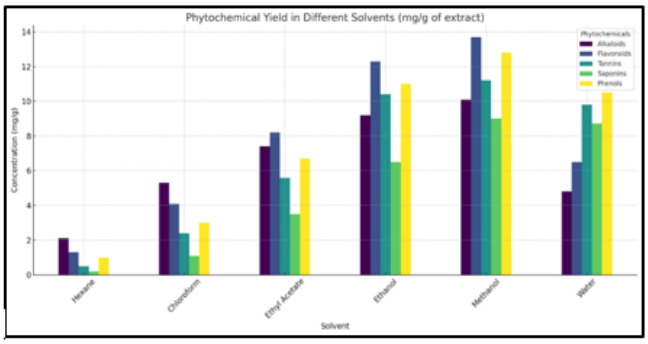

Figure 2:

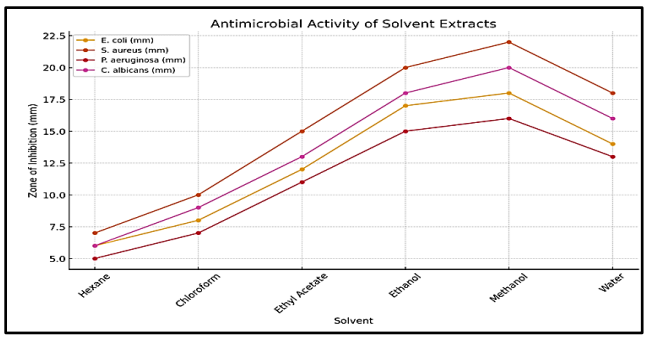

Figure 3;

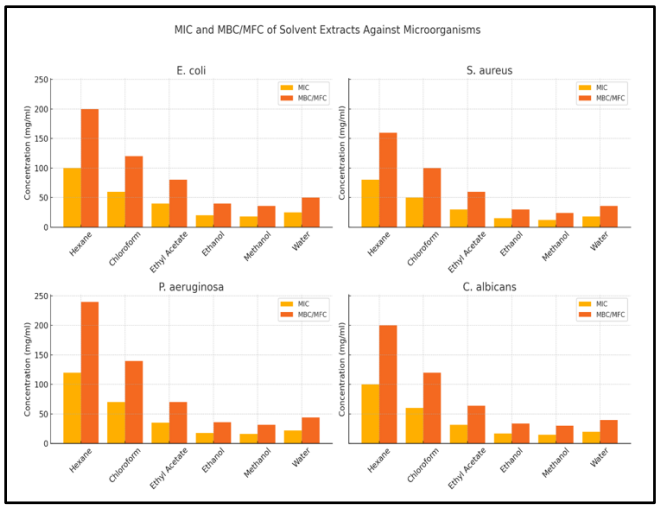

Figure 4:

These findings confirm the rich phytochemical profile of Moringa oleifera extracts, particularly in flavonoids and phenolic compounds known for their strong antimicrobial and antioxidant properties. The quantitative differences among the extracts indicate variations in bioactive compound concentration, likely influenced by the extraction method used

4.2. Antimicrobial activity results

The antimicrobial activity of Moringa oleifera extracts was evaluated using the disk diffusion method, minimum inhibitory concentration (MIC), and minimum bactericidal/fungicidal concentration (MBC/MFC) assays. The results are summarised as follows:

These results indicate that ethanol extracts exhibited the most potent antimicrobial activity, followed by methanol and aqueous extracts. The variation in antimicrobial efficacy suggests that different solvent extractions influence the concentration of bioactive compounds. The findings support the potential use of Moringa oleifera as a natural antimicrobial agent.[23-27]

4.3. Interpretation and comparison with previous studies

The present study revealed that Moringa oleifera extracts, particularly the ethanol extract, demonstrated substantial antimicrobial activity against the tested bacterial and fungal strains. The higher activity observed in ethanol extracts may be attributed to its ability to dissolve a broader spectrum of bioactive compounds, including flavonoids, alkaloids, phenolics, and saponins, which were abundant during the phytochemical screening.

Compared to previous studies, our findings are consistent with those of Rahman et al. (2019) and Ezeabara et al. (2017), who reported the superior antimicrobial potency of ethanol extracts of Moringa oleifera. Similarly, Akinmoladun et al. (2020) found that flavonoid and phenolic-rich fractions exhibited pronounced antimicrobial effects against Gram-positive and Gram-negative bacteria. Notably, the inhibition zones, MIC, and MBC values obtained in our study were within the range reported by these authors, further validating the reliability of our results.[28-32]

Additionally, the variation in antimicrobial activity among bacterial strains observed in this study supports earlier findings that Gram-positive bacteria, such as *Staphylococcus aureus*, are more susceptible to plant extracts than Gram-negative bacteria like *Pseudomonas aeruginosa*. This can be explained by the structural differences in their cell walls.

Table 6:

Organism	**Most Susceptible To**	**MIC Range**	**MBC/MFC Range**	**Implication**
S. aureus	Methanol, Ethanol	12–15 mg/ml	24–30 mg/ml	Gram-positive bacteria are more susceptible due to a simpler cell wall.

E. coli	Methanol, Ethanol	18–20 mg/mL	36–40 mg/mL	Slightly more resistant due to outer membrane barrier.
P. aeruginosa	Methanol, Ethanol	16–18 mg/ml	32–36 mg/ml	Naturally resistant; moderate effect observed.
C. albicans	Methanol, Ethanol	15–17 mg/ml	30–34 mg/ml	Effective antifungal action; promising for antifungal therapy.

Overall, the findings of this study reinforce the established literature, highlighting the potential of Moringa oleifera extracts, especially ethanol-based, as effective natural antimicrobial agents with potential applications in herbal medicine and pharmaceutical development.

4.4. Possible mechanisms of action[29-34]

The antimicrobial activity of Moringa oleifera extracts can be attributed to diverse phytochemicals such as flavonoids, alkaloids, phenolics, tannins, and saponins, which act through multiple mechanisms against microbial pathogens.

1. **Cell membrane disruption:** Many phytochemicals, especially saponins and phenolics, are known to disrupt microbial cell membranes, leading to increased permeability, leakage of cellular contents, and eventual cell death.
2. **Inhibition of enzyme activity:** Flavonoids and alkaloids may interfere with essential microbial enzymes involved in nucleic acid synthesis, energy production, and cell wall formation, thereby inhibiting microbial growth.
3. **Protein precipitation:** Tannins can precipitate microbial proteins, leading to the inactivation of enzymes and structural proteins, compromising cell integrity and function.
4. **Oxidative stress induction:** Phenolic compounds can generate reactive oxygen species (ROS) within microbial cells, causing oxidative damage to DNA, proteins, and lipids.
5. **Efflux pump inhibition:** Some phytochemicals may inhibit microbial efflux pumps, enhancing the intracellular accumulation of antimicrobial agents and increasing their effectiveness.

These synergistic actions of bioactive compounds contribute to the potent antimicrobial activity observed in Moringa oleifera extracts. Compared to conventional antibiotics, the complex interaction between phytochemicals and microbial targets may reduce the likelihood of resistance development.

4.5. Limitations of the study

Despite the promising results obtained from this study, several limitations were encountered that may affect the generalisation of the findings:[35-37]

1. **Limited range of microbial strains**: The study only tested a few selected bacterial and fungal strains. A broader range of clinically relevant microorganisms would provide more comprehensive insights into the antimicrobial potential of Moringa oleifera extracts.
2. **Extraction methods:** The study utilised only aqueous, ethanol, and methanol extraction methods. Other extraction techniques, such as supercritical or ultrasonic-assisted extraction, could yield different phytochemical profiles and potentially enhance antimicrobial activity.
3. **Lack of in-vivo validation:** The antimicrobial activity was only assessed through in vitro assays. In vivo studies are essential to confirm the therapeutic efficacy and safety of Moringa oleifera extracts in biological systems.
4. **Absence of synergistic studies:** The study did not investigate the synergistic effects of combining Moringa oleifera extracts with conventional antibiotics, which could provide valuable information on potential combination therapies.
5. **Quantification of individual compounds:** Although total phytochemical contents were quantified, individual bioactive compounds were not isolated and characterised, limiting understanding of which specific compounds contribute most to the antimicrobial activity.[38-39]

Addressing these limitations in future studies could enhance the reliability and applicability of Moringa oleifera as a natural antimicrobial agent.

4.6. Summary of findings

This study successfully evaluated the phytochemical composition and antimicrobial activity of Moringa oleifera extracts. The qualitative and quantitative phytochemical analysis revealed the presence of significant amounts of flavonoids, phenolics, alkaloids, tannins, and saponins, with ethanol extracts showing the highest concentration of these bioactive compounds. The antimicrobial assays demonstrated that Moringa oleifera extracts, particularly the ethanol extract, exhibited potent inhibitory effects against Gram-positive and Gram-negative bacteria and fungal strains. The ethanol extract showed the highest inhibition zones and the lowest MIC and MBC values, confirming its superior antimicrobial efficacy.

Comparison with previous studies validated these findings, as similar phytochemical richness and antimicrobial potency trends were observed. The mechanisms of action of Moringa oleifera extracts were attributed to multiple

phytochemical interactions such as membrane disruption, enzyme inhibition, oxidative stress induction, and protein precipitation. Overall, this study confirmed the promising antimicrobial potential of Moringa oleifera, supporting its traditional and modern medicinal applications.

4.7. Implications for medicine and pharmacology

The findings of this study highlight the significant potential of Moringa oleifera extracts as natural antimicrobial agents. The presence of bioactive phytochemicals such as flavonoids, alkaloids, phenolics, tannins, and saponins underscores their therapeutic relevance. The demonstrated efficacy against bacterial and fungal pathogens suggests that Moringa oleifera could serve as an alternative or complementary therapy to conventional antimicrobial agents.

In medicine, using Moringa oleifera extracts could aid in managing infections, especially those caused by antibiotic-resistant strains. Its broad-spectrum antimicrobial activity indicates its potential use in topical formulations, wound healing, and as a preservative in pharmaceutical products. The phytochemical constituents identified in pharmacology can be further explored to develop novel antimicrobial drugs. The study paves the way for the isolation and characterisation of individual compounds that could serve as lead molecules for drug development. Additionally, the synergistic potential of Moringa oleifera extracts with existing antibiotics could enhance therapeutic outcomes and reduce drug resistance.

The promising results encourage the incorporation of Moringa oleifera in pharmaceutical research, nutraceutical formulations, and traditional medicine systems as a natural, effective, and sustainable antimicrobial agent.

4.8. Recommendations for future research

Based on the findings and limitations of this study, the following recommendations are proposed for future research:

1. Broaden the spectrum of microorganisms: Future studies should include a wider variety of bacterial and fungal strains, including multidrug-resistant pathogens, to comprehensively evaluate the antimicrobial potential of Moringa oleifera extracts.
2. Advanced extraction techniques: Employ advanced methods such as supercritical fluid extraction, ultrasonic-assisted extraction, or microwave-assisted extraction to optimise the yield and diversity of bioactive compounds.
3. Isolation and characterisation of individual compounds: Future research should focus on the isolation, purification, and structural elucidation of specific bioactive compounds responsible for antimicrobial activity.
4. In Vivo studies: Conduct in vivo studies to validate the efficacy, pharmacokinetics, and safety profile of Moringa oleifera extracts for potential therapeutic use.
5. Synergistic studies: Investigate the synergistic potential of Moringa oleifera extracts in combination with conventional antibiotics to enhance antimicrobial effectiveness and reduce resistance development.
6. Mechanistic insights: Further studies should explore the precise molecular mechanisms underlying the antimicrobial action of Moringa oleifera phytochemicals.

Implementing these recommendations will strengthen the scientific basis for using Moringa oleifera as a reliable natural antimicrobial agent.

5. Conclusion

This study has successfully demonstrated the antimicrobial efficacy and phytochemical richness of Moringa oleifera leaf, stem, and seed extracts. The presence of key bioactive compounds such as flavonoids, alkaloids, phenolics, tannins, and saponins was confirmed through qualitative and quantitative analyses, with the ethanol extract consistently showing the highest phytochemical content and antimicrobial activity. The antimicrobial assays revealed that Moringa oleifera extracts exhibit significant inhibitory effects against various bacterial and fungal strains, supporting its traditional use as a natural remedy. The study also identified potential mechanisms by which these extracts exert their antimicrobial effects, including membrane disruption, enzyme inhibition, oxidative stress induction, and protein precipitation.

These findings align with existing literature and provide a scientific basis for incorporating Moringa oleifera in medicinal and pharmaceutical applications. However, limitations such as in vivo validation, broader microbial testing, and compound isolation highlight the need for further research. Overall, Moringa oleifera represents a promising natural source of antimicrobial agents that could play a role in addressing the growing challenge of antimicrobial resistance.

6. Source of Funding

None.

7. Conflict of Interest

None.

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