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Effects of Organic Moringa Seed Oil Extraction Methods on Antibacterial and Antifungal Properties

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Abstract

Moringa oleifera seed oil (MSO) is widely recognized for its medicinal properties, particularly its antimicrobial potential. This study investigates the influence of extraction methods hot extraction (MOH), cold extraction (MOC), and a control on the antibacterial and antifungal activities of MSO. Antibacterial assays revealed that the MOC extract exhibited the broadest and most potent activity, notably against *Escherichia coli* (15.5 mm inhibition zone) and methicillin-resistant *Staphylococcus aureus* (MRSA) (23.75 mm), outperforming MOH and the control. Conversely, MOH and the control showed more potent activity against *Enterococcus faecalis*, with inhibition zones of 14.5 mm and 24.5 mm, respectively. Overall, MSO demonstrated greater efficacy against Gram-positive bacteria (14.5–24.5 mm) than Gram-negative strains (13–25 mm), with statistically significant differences observed for *E. coli* and *MRSA* across extraction methods. Antifungal evaluations showed minimum inhibitory concentrations (MIC) ranging from 12.5 to 50 mg/mL, depending on the fungal isolate. While MOC and the control exhibited similar antifungal profiles, MOH displayed variable activity, reduced against *Aspergillus flavus* (MIC = 50 mg/mL) and enhanced against Trichoderma sp. These findings underscore the critical role of the extraction technique in modulating the antimicrobial and antifungal efficacy of Moringa seed oil, supporting its potential application as a natural therapeutic agent.

Keywords: Effects, Methods, Water based extraction, Moringa seed oil, Antibacterial, Antifungal Properties, Bioactive compounds.

Introduction

Plant-derived oils have garnered increasing scientific interest due to their complex phytochemical profiles, favorable safety margins, and potent antimicrobial activities.[1] Among these, Moringa oleifera, a fast-

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growing, drought-resistant tree native to South Asia and widely cultivated across Africa, particularly in Niger, stands out for its extensive use in traditional medicine and nutritional applications.[2,3]

All parts of the Moringa plant, including leaves, flowers, seeds, and pods, are valued for their nutritional richness and therapeutic potential. They are commonly incorporated into food formulations, traditional pharmacopoeia, and even industrial products. The essential oils and extracts derived from medicinal plants, such as Moringa, have attracted considerable attention for their broad-spectrum antimicrobial and antifungal properties, primarily due to their abundance of bioactive compounds, including phenolics, terpenoids, and flavonoids.[4,5,6]

Moringa seed oil, in particular, is recognized for its therapeutic efficacy in managing various pathological conditions and is frequently used in the formulation of pharmaceutical and cosmetic products.[7] However, the quality, yield, and bioactivity of this oil are highly influenced by the extraction techniques employed. Despite its growing popularity, there remains a significant gap in the literature regarding how different extraction methods affect the antimicrobial and antifungal potency of Moringa seed oil.

This study aims to evaluate the influence of various extraction methods on the antimicrobial and antifungal activities of Moringa seed oil. By identifying the most effective techniques, this research seeks to optimize the therapeutic potential of Moringa oil and contribute to the development of natural alternatives to synthetic antimicrobial agents.

Materials And Methods

Materials

Samples of Moringa Seed Oil (MSO) extracted using both hot and cold processes were collected from the *Kanambakaché* organic moringa processing unit in the Sahel region of Maradi, Niger. The control sample of Moringa seed oil was purchased from a Moringa oil processing unit in a local market in Maradi, Niger. The three samples were transported to the laboratory for analysis.

The Gram-positive and Gram-negative bacterial strains obtained from the laboratory (ABU, Zaria, Nigeria) were used to assess antimicrobial activity against various Moringa seed oil extracts. The Gram-positive bacteria used were: *Staphylococcus aureus* (*S. aureus*), *Enterococcus faecalis* (*E. faecalis*), methicillin-resistant *Staphylococcus aureus* (MRSA), and *Enterococcus faecium* (*E. faecium*), and one Gram-negative bacterium: *Escherichia coli* (*E. coli*). For the fungal isolates, four strains were used for the fungal analyses: *Aspergillus niger*, *Aspergillus flavus*, Trichoderma sp., and *Candida albicans*.

Authentication of Plant Materials

The collected plant seed materials were taxonomically authenticated by Dr. Karim Saley, a renowned botanist from the Department of Biology, Faculty of Science and Technology, Dan Dicko Dankoulodo University of Maradi. Authentication was confirmed by comparative morphology with standard herbarium references, and voucher specimens were prepared and deposited in the institutional laboratory for archival purposes. The assigned voucher numbers were 2025/BIO/FST003. These references serve as a permanent record for future scientific verification and continuity of research.

Preparation of the Moringa Oil Extracts

The hydro-methanol was used to extract a solution of Moringa oil. For this, 100 ml of defatted Moringa seed oil was mixed with 600 ml of water-methanol solvent (20:80) in a 1000 ml beaker. The mixture was left to macerate for 96 hours (4 days) at room temperature (25°C). After the maceration period, the solution was filtered under vacuum through Whatman No. 1 filter paper. The resulting filtrate was concentrated until the solvent was removed entirely. The resulting dry extract was stored in vials until further use.[8]

Preparation of Culture Media

A nutrient agar medium was used for the isolation and maintenance of bacterial strains. The preparation of this culture medium involved dissolving 15.2 g of Mueller-Hinton Agar (MHA) powder in 400 mL of

distilled water, then autoclaving at 121°C for 15 minutes. This sterilization process ensures the destruction of contaminating microorganisms and facilitates proper agar dissolution.[1]

The molten agar was cooled to approximately 50–60°C to avoid damaging the nutrients and prevent excessive condensation. The cooled medium was then poured into sterile 20 ml Petri dishes. Aseptic conditions must be maintained to preserve sterility and ensure uniformity of the culture medium surface.[6] The prepared plates were incubated at 37°C for 24 hours to allow excess moisture to evaporate, producing dry, firm surfaces suitable for bacterial inoculation.

Preparation of Bacterial Solutions

Bacterial cultures were grown in Lysogeny Broth (LB) medium, and the pathogenic bacterial isolates (*S. aureus, E. faecalis, E. coli, and MRSA*) were inoculated into separate tubes containing 20 ml of sterile LB broth. These inoculated tubes were incubated overnight at 37°C with shaking in a shaker incubator to promote aeration and ensure uniform bacterial growth. The shaking process facilitates aeration of the culture, promoting vigorous bacterial proliferation.[9] Agar diffusion method, or "well diffusion method": This is the basic technique used to study a substance's ability to exert an antimicrobial effect. It is also called the "agar dilution" technique for determining the activity of extracts. Wells (approximately 6 mm in diameter) are made in the agar, into which a quantity of pure or diluted essential oil is poured; after incubation, zones of bacterial growth inhibition are obtained (for active oils) and measured.

Tests of Antibacterial and Antifungal Activities

To determine the antibacterial activity of the MSO extracts, which is indicated by the appearance of inhibition zones (IZ), the diameter of the inhibition zone around each disc was measured using a ruler.[10] The larger the diameter of this zone, the more sensitive the bacterial strain.[11,12]

To determine the antifungal activity of the tested MSO extracts (fungicidal or fungistatic), the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) were determined. If the fungus resumes growth, the activity is fungistatic; otherwise, it is considered fungicidal.

Statistical Analysis

XLSTAT version 1.3 and SPSS software were used to determine the means ± standard deviation and perform analysis of variance (ANOVA). Multiple pairwise comparisons and Fisher's test at a 5% significance level were used to analyze the effects of the oil extraction method on the antibacterial properties.

Results

Antibacterial Activities of Moringa Seed Oil

The antibacterial activity of three types of Moringa Seed Oil extracts: Cold-Pressed Moringa Seed Oil (MOC), Hot-Pressed Moringa Seed Oil (MOH), and the control, was evaluated against five (5) types of standard clinical bacterial isolates: *E. coli*, *E. faecalis*, *S. aureus*, *MRSA*, and *E. faecium*.

The antibiogram results showed that the MOC extract exhibited the broadest spectrum of activity. The MOC extract was most effective against Gram-negative bacteria, with inhibition zones (IZ) of 15.5 mm against *E. coli* and 23.75 mm against MRSA.

Conversely, the MSO extract and the control sample demonstrated more specific and compelling activity against three bacterial strains (Table 1). The MOH extracts and the control sample demonstrated specific antibacterial activity against *E. faecium.* In contrast, the MOC extract showed no activity against this bacterium. The control extract showed antibacterial activity against *E. faecalis* and *S. aureus* but was ineffective against *MRSA* and *E. coli*.

Table 1: Antibacterial activity of Moringa Seed Oils (MSO)

Isolates	Inhibition zone (IZ) in mm		
	MOC	MOH	Control
E. coli	15.50±0.71	0±0.00	0±0.00
E. faecalis	22.25±0.90	13.50±0.43	24.50±1.30
S. aureus	17.50±0.76	15.25±0.70	18.25±0.8
MRSA	23.75±1.10	0±0.00	0±0.00
E. faecium	0±0.00	14.50±0.71	13.50±0.71
DMSO	0±0.00	0±0.00	0±0.00

MOC: Moringa Seed Oil Cold extract; **MOH**: Moringa Seed Oil Hot extract; **DMSO**: Dimethyl Sulfoxide; **MRSA** : Methicillin-resistant *Staphyloccocus aureus*.

The comparative analysis of the inhibition zones (Figure 1) shows the different spectra of action of the MSO extracts. These spectra of action demonstrated that the MOC extract possesses better antibacterial activity than the other extracts.

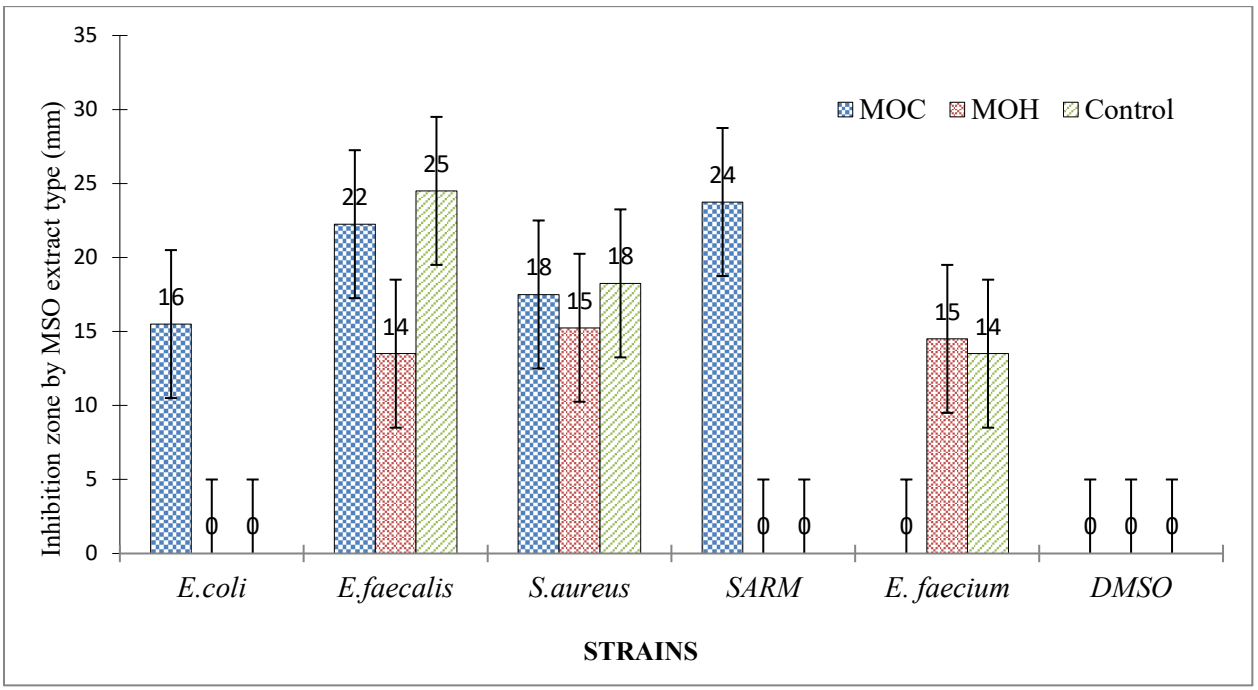

Figure 1: Comparative analysis of bacterial inhibition zones as an effect of Moringa seed oil (MSO) extracts.

Effects of Oil Extraction Methods on Antibacterial Properties

Analysis of the mean inhibition zones enabled evaluation of the effects of Moringa seed oil (MSO) extraction methods on bacterial isolates, demonstrating a significant impact on antibacterial properties (Table 2). However, despite considerable variations in mean inhibition zones across the different Moringa seed oil extracts, statistical analyses using Fisher's tests at $\alpha = 0.05$ showed that the treatments applied to *E. coli* and *MRSA* are significantly different. For the other treatments, there was no significant difference between the MSO extraction methods. MOC showed substantially higher antibacterial activity than the other ones.

Table 2: Effect of the Moringa Seed Oils extraction method on antibacterial properties

Extraction method	*Escherichia coli*	*Enterococcus faecalis*	*Staphylococcus aureus*	*MRSA*	*Enterococcus faecium*
MOC	15.50±8.94[a]	22.25±2.13[a]	17.50±0.59[a]	23.75±13.70[a]	0[a]
MOH	0[b]	13.50± 1.59[a]	15.25±0.32[a]	0[b]	14.50±3.93[a]
Control	0[b]	24.50±2.13[a]	18.25±0.62[a]	0[b]	13.50±3.88[a]
Pr > F (model)	Pr = 0.0001	Pr = 0.685	Pr = 0.618	Pr = 0.0001	Pr = 0.618
Significant effect	YES	NO	NO	YES	NO

MOC: Moringa Seed Oil Cold extract; **MOH**: Moringa Seed Oil Hot extract; **DMSO**: Dimethyl Sulfoxide; **MRSA** : Methicillin-resistant *Staphyloccocus aureus*.

Evaluation of the Antifungal Activities of HGM

Antifungal tests performed on Moringa seed oil extracts (Table 3) revealed that the antifungal profiles of the extracts varied depending on the fungal strains. MOC exhibited the same profile as the control on all four strains tested. MOH showed a variable profile across the strains. MOH exhibited antifungal activities identical to the control against the *A. niger* and C. albicans strains, while the antifungal activity was reduced against *A. flavus* (MIC = 50 mg/ml). All treatments showed efficacy MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration (MIC = 25 mg/mL, MFC = 50 mg/mL) against the *A. niger* strain, but there was no significant difference in antifungal activity among the treatments.

Table 3: Antifungal activity of Moringa Seed Oil extracts

Isolates	**MOC (mg/mL)**		**MOH (mg/mL)**		**Control (mg/mL)**	
	MIC	**MFC**	**MIC**	**MFC**	**MIC**	**MFC**
Aspergillus niger	25	50	25	50	25	50
Aspergillus flavus	25	50	50	0	25	50
Trichoderma sp	25	50	12.5	25	25	50
Candida albicans	25	50	25	50	25	50

MOC: Moringa Seed Oil Cold extract; **MOH**: Moringa Seed Oil Hot extract; **MIC**: Minimum Inhibitory Concentration; **MFC**: Minimum Fungicidal Concentration

Discussion

The present study highlights the potent antimicrobial properties of Moringa oleifera seed oil, particularly its pronounced antibacterial activity against Gram-positive bacteria, such as Staphylococcus aureus (MRSA) and Enterococcus faecalis, compared to Gram-negative bacteria, such as *Escherichia coli*. This trend is reflected in the inhibition zone diameters, which ranged from 15 to 25 mm for Gram-positive bacteria and 13 to 25 mm for *E. coli*. The relatively lower susceptibility of Gram-negative bacteria is attributed to their unique cell envelope architecture, notably the outer membrane, which is enriched in lipopolysaccharides. This structure acts as a selective permeability barrier and is further reinforced by active efflux pumps that expel antimicrobial agents, thereby reducing intracellular accumulation. [13,14]

The extraction method played a critical role in modulating the antimicrobial efficacy of Moringa seed oil. Cold extraction preserves bioactive constituents: A study comparing chemical and physical cold extraction methods found that cold-extracted Moringa oil retained higher levels of sensitive phytochemicals, including flavonoids and terpenoids, known for their antimicrobial properties.[15] These compounds are known for their broad-spectrum antimicrobial properties and are distributed throughout various tissues of M. oleifera, which contains over a hundred phytochemicals.[16] Variability in antimicrobial potency may also stem from factors such as the specific plant organ used, the maturity stage at harvest, and the extraction conditions, all of which influence the profile and concentration of secondary metabolites.[17]

The antifungal evaluation further supports the therapeutic potential of Moringa seed oil. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) assays revealed significant inhibitory effects against fungal pathogens, including *Aspergillus niger*, *A. flavus*, Trichoderma sp., and *Candida albicans*. MIC values ranged from 12.5 to 50 mg/mL, indicating moderate to vigorous antifungal activity depending on the strain and extract type. Notably, *C. albicans* exhibited the highest sensitivity, with MIC values as low as 12.5 mg/mL. This heightened susceptibility aligns with previous findings that essential oils rich in phenolic and terpenoid compounds are particularly effective against yeast-like fungi.[18,19]

In contrast, *A. niger* demonstrated greater resistance, with MIC values ranging from 25 to 50 mg/mL. This observation is consistent with the robust cell wall structure and adaptive resistance mechanisms of Aspergillus species, which confer reduced vulnerability to phytochemicals.[20] A. flavus and Trichoderma sp. showed intermediate sensitivity, with MIC values around 25 mg/mL, suggesting a relatively uniform response across extracts.

The distinction between MIC and MFC values provides insight into the mode of action of Moringa seed oil. For *C. albicans*, the proximity of MIC and MFC values (12.5–25 mg/mL) suggests a fungicidal effect at low concentrations. Conversely, higher MFC values for Aspergillus species imply a primarily fungistatic mechanism, where growth is inhibited without complete eradication. These differences may be attributed to variations in fungal membrane permeability and enzymatic detoxification pathways, which influence the efficacy of bioactive compounds.[21]

Conclusion

This study confirms the potent antimicrobial and antifungal properties of *Moringa oleifera* seed oil, highlighting its potential as a natural alternative to synthetic agents in combating microbial resistance. The efficacy of Moringa seed oil is closely linked to its rich phytochemical composition, including flavonoids, terpenoids, and isothiocyanates, and is significantly influenced by the extraction method employed. Notably, the cold-extracted MOC variant demonstrated superior antimicrobial activity, especially against Gram-positive bacteria such as *MRSA* and *E. faecalis*, suggesting its suitability for targeted therapeutic applications. The differential sensitivity of microbial strains, particularly the heightened susceptibility of *Candida albicans* and the relative resistance of *Aspergillus niger*, underscores the importance of pathogen-specific approaches when evaluating plant-based antimicrobials. These findings support the strategic use of Moringa seed oil in the development of phytotherapeutic formulations, especially in regions with high antimicrobial resistance burdens and limited access to conventional treatments. Future research should explore the synergistic effects of Moringa seed oil with other natural or synthetic agents, assess its safety profile in vivo, and investigate its potential integration into food preservation, dermatological products, and agricultural biocontrol strategies.

Conflict of Interest

By this declaration, the authors of this manuscript affirm that there is no conflict of interest of any kind, either between the authors themselves or between the authors and third parties.

Authors' Contributions

Mr. Massaoudou Mahamane: conducted the literature review, collected samples for laboratory analysis, performed data analysis and interpretation, and wrote the initial draft. Dr. Issoufou Amadou: provided conceptualization, supervision, validation, review, and editing, and offered advice on structuring the data analysis and refining the manuscript. Dr. Ibrahim Halilou Amadou: supported the activities and reviewed the work; and Mr. Mahamadou Rabiou Moudi Aboubacar: facilitated the laboratory work, data collection, and co-wrote the draft.

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