Original Research Article
ISSN: 2583-7419
Journal homepage: biologyforum.actabotanica.org

Phytochemical Extraction and Effects of Solvent on Antimicrobial Potential of *Moringa Oleifera* Seeds Extracts

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Citation: Terngu Paul Ugosor, Apeyuan Kparev-Wua David and Naku Julius Uko (2026). Phytochemical Extraction and Effects of Solvent on Antimicrobial Potential of *Moringa Oleifera* Seeds Extracts. *Acta Biology Forum*.
DOI: https://doi.org/10.51470/ABF.2026.5.1.13

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Received 11 January 2026 | Revised 14 February 2026 | Accepted 12 March 2026 | Available Online 04 April 2026

ABSTRACT

The effect of extraction solvent on phytochemical recovery and antimicrobial activity remains a critical factor in medicinal plant research because of rising antimicrobial resistance worldwide. Moringaoleifera seeds are known to contain diverse bioactive phytochemicals with antimicrobial properties. This research examined how solvent choice influences phytochemical extraction recovery and antimicrobial activity of M. oleifera seed extracts using water, methanol, ethanol, and ethyl acetate. Seeds were air-dried, pulverized, and subjected to cold maceration in respective solvents. Extracts were concentrated and screened for phytochemicals using standard qualitative methods. Bioactivity was evaluatedagainst Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus , and Candida albicansusing broth microdilurionfollowing Cinical and LaboratoryStandard Institute (CLSI) M07 gudelines. Extraction yield result revealed that methanol generated the highest extraction (18.73 ± 0.21), followed by ethanol (16.93± 0.20), water (12.40± 0.26), while ethyl acetate yielded the least (10.27± 0.15). Qualitative phytochemical analysis showed terpenoids, alkaloids, steroids,flavonoids, glycosides tannins, saponins, and phenolics. Methanolic extract contained maximum Total PhenolicContent, TPC (85.40 ± 2.10 mg GAE/g), Total Flavonoid Content, TFC (62.32 ± 1.80 mg QE/g), and Tannins (41.20 ± 1.50 mg/TAE/g) and exhibited the most potent antimicrobial activity with least MIC and MCM/MFC of 12.5 mg/mL and 25.0 mg/mL, respectively. Among the organisms, Staphylococcus aureus and Candida albicanswere more susceptibleto the extracts. ANOVA indicated significant differences ($p < 0.05$) across extract concentrations, with mean separation by Tukey'sHonestly Significance Difference (HSD) Test. Methanol extract demonstrated maximum bioactivity, followed by ethanol, ethyl acetate, and water. The findings confirmed that solvent polarity significantly influences phytochemical composition and antimicrobial efficacy of M. oleifera seed extracts. Polar organic solvents, particularly methanol and ethanol are more efficient for recovering antimicrobial bioactive compounds from M. oleifera seeds, suggesting their suitability for pharmaceutical applications.

Keywords: *Moringaoleifera, phytochemical extraction, solvent polarity, antimicrobial activity, seed extract, bioactive compounds.*

1.0 Introduction

1.1 Background of the Study

Plants have historically served as rich reservoirs of biologically active compounds exhibiting broad therapeutic properties.*Moringaoleifera*Lam, known as drumstick tree or miracle tree, has attracted significant scientific research due to its rich reservoir of phytochemicals and broad spectrum antimicrobial. The plant is native to India and is widely cultivated in Africa [1] [2]. *M. oleifera* is ued in traditional medicine for its nutritional and pharmacological benefits,where it is traditionally used for treating infections, inflammations, and malnutrition. The seeds of *M. oleifera* extracts are rich in bioactive phytochemicals with therapeutic relevance [3]. Bioactive constituents of the plant haveantimicrobial, anti-inflammatory, antioxidant,and anticancer properties [4] [5] [6] [7].

With increasing antimicrobial resistanceamong pathogenic microorganisms, plant-derived antimicrobials have emerged as promisingnatural alternatives tosynthetic antibiotics. *M. oleifera* presents itself as a promising substitute to synthetic antimicrobial agents due to its rich phytochemicals. Different parts of the plant (leaves, flowers, seeds, bark, and roots) contain secondary metabolites and bioactive compouns such as flavonoids, alkaloids, tannins, saponins, phenolic, isothiocynates, glucosinolates, sterols, fatty acids (e.g., oleic acid), and terpenoids that contribute to anti-inflammatory, antioxidant, and antimicrobial properties[8] [9] [10].According to [11] and [12],*M. oleifera* seeds contain significant amounts of lipids (30 - 40 %), proteins, and phenolic compounds that contribute to antimicrobial and antioxidant effects[13].

Several studies have underscored solvent-dependent variations in antimicrobial activity. For example, extracts prepared with polar organic solvents often show enhanced inhibitory effects when compared with aqueous extracts as reported by [14] [15]. Conversely, aqueous extracts, while safer and more environmentally sustainable, may yield lower antimicrobial efficacy due to limited extraction of non-polar bioactive constituents.

Ethyl acetate extracts have also been reported to possess unique antimicrobial profiles due to selective enrichment of moderately polar secondary metabolites, as recorded by [16]. Ethanol, methanol, and ethyl acetate extracts show higher antimicrobial activity against *S. aureus*, moderate activity against *E. coli*, and variable activity against *P. aeruginosa*[17]. Enhanced efficacy of ethanol, methanol, and ethyl acetate extracts is largely attributed to their ability to extract membrane-active compounds capable of disrupting lipid bilayers [18] [19] [20] [21].

Despite the growing body of research on *M. oleifera*, comparative analyses of extraction solvents remain incomplete. Comprehensive evaluation of water, methanol, ethanol, and ethyl acetate in relation to extract yield, phytochemical amount, and antimicrobial efficacy against clinically relevant pathogens can provide useful insights on solvent effects and guide the selection of optimal extraction strategies.

This research aims to evaluate phytochemical composition and bioactivity potential of *Moringaoleifera* seed extracts obtained with water, methanol, ethanol, and ethyl acetate. By correlating solvent polarity with extract chemistry and antimicrobial activity, the research seeks to identify solvent systems that maximize the therapeutic potential of *M. oleifera* seed extracts. The findings are expected to promote development of plant-derived antimicrobial agents and validate evidence-based utilization of *M. oleifera* in traditional and modern medicine.

Figure 1: Moringaoleifera(a) Plant(b) Pods,and © Seeds

1.2 Traditional and Medicinal Values of*Moringaoleifera*

Moringaoleifera, commonly knownas the "miracle tree," is utilized in traditional medicine and nutrition across Africa, Asia, and other tropical regions. Nearly all parts of the plant (flowers,leaves, seeds, pods, roots, bark , stems) has distinct health-promoting properties attributed to its rich phytochemical constituents, vitamins, and essential minerals[22] [23].

The leaves are the most commonly used part of *M. oleifera* and are highly valued both nutritionally and medicinally. The leaves are widely consumed as vegetables in soups, sauces, and teas. In many communities, the leaves are used to manage malnutrition, especially among nursing mothers and children due to their high protein content and abundance of micronutrients like iron, calcium, and vitamins A and C[1] [3] [24].

Medicinally, the leaves possess strong antioxidant properties due to compounds like quercetin and chlorogenic acid, which help scavenge free radicals and reduce oxidative stress[10] [25]. They are traditionally used in managing conditions such as anemia, hypertension, and diabetes[2]. Their hypoglycemic effect is linked to increased insulin sensitivity and decreased blood glucose levels[26]. Additionally, the leaves exhibit antimicrobial and anti-inflammatory activities, useful in treating infections, wounds, and inflammatory disorders. They are also known to support liver function and enhance immune response.

The seeds of *Moringaoleifera*are multifunctional and hold both environmental and medicinal importance. One of the most notable traditional applications is in water purification. Crushed seeds act as natural coagulants, binding suspended particles and reducing microbial contamination in water[27] 28] [29] [30] [31]. This practice is especially valuable in rural areas lacking access to clean drinking water. The seeds contain bioactive compounds with antimicrobial properties, making them effective against many pathogens. They are used in managing digestive disorders such as constipation and indigestion. The oil extracted from seeds, commonly known as ben oil, is rich in oleic acid and is applied topically for skin hydration, treatment of skin infections and wound healing [3] [12]. *M. oleifera*seeds also demonstrate anti-inflammatory and antioxidant effects, contributing to their role in managing chronic diseases.

The flowers of the plant are edible and have both nutritional and therapeutic significance. Traditionally, the flowers are often cooked or infused as herbal tea and are believed to improve vitality and reproductive health[1] [3]. In some cultures, the flowers are used as a natural remedy to enhance libido and support hormonal balance[11]. The flowers contain flavonoids and other phytochemicals that contribute to their anti-inflammatory and antioxidant activities[31]. They are traditionally used in treating respiratory conditions such as colds and coughs. Additionally, flower extracts have mild diuretic properties, which help in managing urinary tract issues and promoting kidney health. Some studies[1] [9] [25] [32] also suggest their role in supporting cardiovascular health.

The immature pods of *Moringaoleifera*, commonly called drumsticks, are widely cooked and consumed as food because of their pleasant taste and high nutritional value. The pods are rich in dietary fiber, essential amino acids and vitamins, beneficial for digestive health[31]. Medically, the pods are known to aid digestion and prevent constipation due to their fiber content[33] [34]. They also contribute to cholesterol regulation and cardiovascular health. Traditionally, they are used to strengthen bones and improve joint health because of their mineral content, particularly calcium and phosphorus[35] [36]. Additionally, they possess mild antimicrobial properties and support general body nourishment as recorded by [1] and [37].

The roots of *Moringaoleifera* resemble horseradish in taste and are used cautiously in traditional medicine. The roots are used as stimulants and digestive aids, often in small quantities[1]. According to [9], some traditional practices apply the root preparations to relieve pain and inflammation, particularly in cases of rheumatism and arthritis. The roots contain alkaloids and other bioactive constituents with anti-inflammatory analgesic effects[34]. As recorded by [1], the roots are sometimes used to treat edema and digestive disorders. However, the roots also contain potentially toxic substances, such as spirochin, which can pose health risks if consumed excessively[25]. Therefore, their medicinal use requires careful dosage and expert guidance.

The stems of the plant are less commonly used medicinally but still have some traditional relevance. They are primarily utilized as fuelwood and for making simple tools or fencing materials. In some cases, stem extracts are used in folk medicine[11]. Medicinally, stem extracts may exhibit mild anti-inflammatory and antimicrobial properties[1] [11], although their medicinal significance is less pronounced compared to other parts of the plant. Stem extract may also contribute to general wellness when used in combination with other plant parts.

The bark of *M. oleifera* is used in ethnomedicine for treating microbial infections and woud healing [11].

According to [1] and [11], the bark is often prepared as decoctions or infusions to treat digestive issues, infections, and general weakness. The bark is also used in some cultures as a remedy for fever and pain, as reported by [31]. The bark possesses astringent, antimicrobial, and anti-inflammatory properties because of tannins and other secondary metaboloites and is used in managing diarrhea, dysentery, and gastrointestinal disorders[1] [3] [11]. Additionally, bark extracts may help in reducing inflammation and alleviating pain[11]. Traditional applications as recorded by [9]also include its use as a cardiac stimulant and circulatory tonic.

Moringaoleifera is a highly versatile plant with significant traditional and medicinal importance. Its various parts contribute uniquely to health promotion, ranging from nutritional supplementation to disease management. While many of its uses are supported by scientific studies, some parts, particularly the roots and bark,should be used with caution due to potential toxicity. Overall, theplant remains a useful natural resource in both indigenous healthcare systems and advanced pharmacological research.

1.3 Research Problem

The increase in drug multi-resistant variant of pathogens globally has promtedthe search for natural antimicrobial agents. Although, the extracts of *M. oleifera* have shown inhibitory effects against many bacterial and fungal pathogens, suggesting potential applications in pharmaceutical, food preservation, and public health sectors, variability in reported antimicrobial efficacy highlights the importance of extraction methodology, especially solvent selection as a determinant of bioactivity.

Phytochemical extraction is a critical step in isolating bioactive constituents from plant matrices. The efficiency of extraction, the profile of compounds obtained, and biological activity are strongly influenced by the choice of solvent. Solvents differ in polarity, solubility parameters, and chemical affinity for specific classes of phytochemicals, which in turn determines both the qualitative and quantitative composition of the extract. Understanding solvent effects is therefore essential for optimizing extraction protocols that target compounds with antimicrobial potential.

This research aims to address these gaps by investigating the effects of solvent type as a determinant of bioactivity. The research will add to theexisting body of knowledge, validate, and provide a scientific basis for the potential use of *Moringaoleifera*seeds in the pharmaceutical, food, and medical sectors of the economy.

1.4 Aim and Objectives of the Study

The aim of the study is to investigate the effect of solventas a determinant of bioactivityof *moringaoleifera* seeds on selected clinically important pathogens.

The specific objectives include:

1. Qualitative and quantitative phytochemical screening of *Moring oleifera* seeds extracts.
2. Evaluate the antimicrobial efficacy of *Moringaoleirera* seeds extract against selected clinically important pathogens.
3. Compare the bioactivity of different extracts: methaonol, ethanol, ethyl acetate, and water.
4. Determine MinimumInhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) against the isolates.
5. Contribute to development of natural antimicrobial agents from plant sources as alternatives to synthetic antimicrobial agents.

1.5 Significance of the Study

This research provides valuable scientific and practical insights into the phytochemical constituents and antimicrobial potential of *M. oleifera* seed extracts, with particular emphasis on the influence of extraction solvents on bioactivity. By evaluating different solvents such as methanol,water, ethanol, and ethyl acetate, the research identifies optimal extraction conditions for isolating bioactive constituents, thereby contributing to the advancement of Phytochemistry and natural product chemistry.

The research findings are relevant in the context of increasing antimicrobial resistance, as they support exploration of plant-based alternatives to conventional antibiotics. Additionally, the study offers potential applications in the pharmaceutical and healthcare sectors by promoting development of cost-effective, accessible, natural, and eco-friendly antimicrobial agents. It also provides a base for further studies on compound isolation, drug formulation, and clinical evaluation, while supporting the sustainable utilization of medicinal plants.

2.0 Review of Related Literature

2.1 Overview of phytochemicals in *Moringaoleifera* seed extracts

Phytochemical screening of *Moringaoleifera* seed extracts using water, hexane, ethanol, methanol, and ethyl acetate as solvents consistently reveals alkaloids, terpenoids, glycosides, saponins, tannins, phenolic compounds, flavonoids, and steroids [2] [8] [10] [31] [33] [37] [38]. These phytochemicals are responsible for the plant's medicinal properties, including inhibition of bacterial and fungal pathogens and protection against oxidative stress [4] [39] [40] [41] [42] [43].

2.1.1 Alkaloids

*Moringaoleifera*seedscontains moderate levels of alkaloids,a classof nitrogen-containing organic compounds. Alkaloids can interfere with DNA replication and disrupt microbial cell membranes , causing inhibition of pathogens such as *S. aureus* and *E. coli*[31]. Inflammatory mediators like prostaglandins and cytokinesalso inhibited byalkaloids, reducing tissue inflammation. In addition, alkaloids are known to initiate apoptosis (programmed cell death) in cancer cells and inhibit tumor growth [40]. Some alkaloids exhibit vasodilatory effects, helping regulate blood pressure[41].

2.1.2 Saponins

Saponins are glycosidic compounds with surfactant properties. Saponinscause leakage of cellular proteins by interacting with microbial membranes, mitigating oxidative stress throughfree radicals scavenging activity, inhibiting inflammatory pathways, and reduce swelling, and decrease cholesterol absorption in the intestine, thereby reducing blood cholesterol levels [31] [41].

2.1.3 Tannins

Tannins are polyphenolic compounds with strong protein-binding ability [42]. Tannins inhibit microbial enzymes and precipitate proteins, limiting microbial growth, neutralize reactiveosygen species(ROS), and prevent lipid peroxidation[31]. Tannins reduce inflammation by stabilizing tissues and inhibiting enzymes involved in inflammation, and inhibit tumor growth by preventing DNA damage and oxidative stress[39].

2.1.4 Phenolic compounds

Phenolics include gallic, chlorogenic, and coumaric acids [8] [31]. Phenolics are among the most powerful antioxidants (scavengers of ROS)) in *moringa*[35]. Phenolics disrupt microbial membranes and inhibit enzyme activity, enhancing antimicrobial activity [41]. They can also suppress pro-inflammatory mediators like TumorNecrosisFactor alpha (TNF-α) and interleukins as well as prevent oxidation of Low-Density Lipoprotein (LDL) cholesterol, thus reducing the risk of atherosclerosis[13] [38] [44].

2.1.5 Flavonoids

Flavonoids (e.g., quercetin, kaempferol) are a major subclass of phenolic compounds[39]. Flavonoids are potent free radical scavengers and protect cells from oxidative stress[41]. They can inhibit bacterial growth and biofilm formation [31]. Flavonoids suppress enzymes such aslipoxygenaseand cyclooxygenase (COX) as well as induce apoptosis and suppress cancer cell proliferation [13]. Compounds like quercetin have shown strong antibacterial and anticancer activities [7]. Flavonoids also improve endothelial function and reduce blood pressure, improving cardiovascular [42].

2.1.6Terpenoids

Terpenoids are lipid-soluble compounds abundant in *moringa* seed oils. These compounds disrupt lipid bilayers and interfere with membrane integrity. Their antimicrobial activity involves damage to microbial cell membranes and inhibition of microbial metabolism [42]. Terpenoids reduce inflammation by modulating immune responses and have anticancer activity by inhibiting tumor cell growth and promotingapoptosis[41]. Terpenoids help regulate blood sugar and cholesterol levels and protect against heart diseases, promoting cardiovascular benefits[39] [31].

2.1.7 Steroids

Plant steroids (phytosterols) are structurally similar to cholesterol (Terngu et al., 2024). Steroids inhibit inflammatory mediators and reduce swelling[9]. Phytosterolshave the ability to alter membrane permeability in microorganisms [40]. Phytosterols reduce cholesterol absorption, lowering LDL levels and preventing heart disease[4]. Some steroids in *Moringaoleifera* have antimicrobial properties as reported by [6].

2.1.8 Glycosides

Glycosides consist of sugar and non-sugar components (aglycones)[41]. Glycosides can interfere with microbial metabolism and enzyme systems[13]. Cardiac glycosides improve heart contractility and regulate heart rhythm, promoting cardiovascular health. Certain glycosides inhibit cancer cell proliferationas recorded by [6] and [19].

Moringaoleifera seed extracts are pharmacologically rich because of various phytochemicals. These bioactive susbstances exhibit synergistic effects, making the seeds potent natural source of antimicrobial agents, antioxidants, anti-inflammatory compounds, anticancer agents,and cardioprotective substances. Alkaloids, tannins, flavonoids, and saponins possessstrong bioactivity against microorganisms like*E.coli*,*Staphylococcus aureus*, and*Pseudomonas aeruginosa*[7][33]. Phenolics and flavonoids reduces oxidative damage through free radicals scavenging , thereby preventing chronic diseases [10]. Phytosterols, flavonoids, and saponins help lower cholesterol, improve blood circulation, and prevent atherosclerosis [2] [17] [20] [31]. The broad-spectrum biological activities, antioxidant defense, anticancer, cardiovascular effects, and reduced inflammatory effects of these phytochemicals justify their increasing application in pharmaceuticals, nutraceuticals, and traditional medicine.

Table 1: Summary of pharmacological properties of Moringaoleifera seeds

Phytochemical	Antimicrobial	Antioxidant	Anti-inflammatory	Anticancer	Cardiovascular
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	+
Tannins	+	+	+	+	+
Phenolics	+	++	+	+	++
Flavonoids	+	++	++	++	++
Terpenoids	+	+	+	+	+
Steroids	+	_	++	+	++
Glycosides	+	_	+	+	++

Key: *++ = Strong activity,+ = Present activity, - = No activity*

2.2 Pharmacological Properties of *Moringaoleifera* Seed Extract

2.2.1Antimicrobial activity

Moringaoleifera seeds have drwanconsiderable scientific research due to their natural broad-spectrum bioactivity against pathogenic bacteria, fungi, and to a lesser extent, viruses. This biological activity is largely due to diverse secondary metabolites such as alkaloids, saponins, flavonoids, phenolics, tannins, terpenoids, and bioactive peptides.

Several researches have revealed that *Moringaoleifera* seeds extracts exhibit antimicrobial effects against both Gram-positive and Gram-negative microorganisms and pathogenic fungi[6] [7] [19] [18] [31]. For example, studies have demonstrated effectiveness of ethanol and methanol leaf extracts of the plant against pathogenicmicroorganisms including *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli,*and *Salmonella typhi*, which are commonly associated with infections and antibiotic resistance challenges[18]. Similar studies [10] [16] and [31] have shown that aqueous extracts have mild antimicrobial effects, highlighting the influence of solvent polarity on bioactivity.

Studies have also revealed that Gram-positive bacteria are generally more vulnerable due to their less complex cell wall structure, whereas Gram-negative bacteria have an outer membrane that restricts penetration of bioactive compounds[19] [33] [38] [39]. However, certain solvent extracts (especially ethanol and ethyl acetate) have shown significant activity against both groups, indicating the presence of potent antimicrobial constituents [16].*Moringa* seed extracts have also shown antifungal activity against *Candida albicans*, suggesting a broad antimicrobial spectrumas reported by [33].

One of the most important recent developments is the demonstrated antimicrobial activity of *Moringaoleifera* seed extracts against antibiotic-resistant strains.

A report by [7] indicated that both seed and leaf extracts of *Moringaoleifera*inhibited drug-resistant clinical isolates, highlighting their potential as alternative antimicrobial agents in fighting antimicrobial resistance. Compounds in *Moringaoleifera* seeds have demonstrated ability to inhibit biofilm formation, disrupt established biofilms, and reduce microbial adhesion to surfaces [14][19] [31] [34] [45]. Biofilm formation is a major virulence factor that enhances microbial resistance to antibiotics. This property is particularly important in managing chronic infections and medical device-associated infections. This suggests that *Moringaoleifera*seed extracts may act as alternative therapeutics, adjuncts to conventional antibiotics, and sources of novel antimicrobial compounds.

Recent advancements have explored the importance of *Moringaoleifera* seed extracts in green synthesis of nanoparticles, particularly silver nanoparticles as reported by [20] [46]. These nanoparticles exhibit improved antimicrobial activity because of increased surface area and improved interaction with microbial cells. Such combinations significantly boost bactericidal efficiency, penetration into microbial cells, and stability of antimicrobial agents.

The antimicrobial properties of *Moringaoleifera* seed extracts have significant implications for healthcare and industry for the development of plant-based antibiotics, use in wound healing and topical formulations, application in water purification (due to antimicrobial peptides),and incorporation into food preservation systems. The natural origin, low toxicity, and broad-spectrum activity make*Moringaoleifera* a promising candidate for sustainable antimicrobial strategies.

2.2.2Antioxidant activity

Moringaoleifera seed extracts possess remarkable antioxidant activities due to high content of phenolics, flavonoids, tannins, and other redox-active constituents. These phytochemicals play a important role in scavenging reactive oxygen species (ROS) and protecting biological systems from oxidative damage[10]. Antioxidant properties of *Moringaoleifera*seed extracts canbe brokendown as follows:

(a) Free radical scavenging mechanisms

Free radical scavengingactivity of *Moringaoleifera*sseedis mainly attributed to the ability of phenolics and flavonoids to release hydrogen atoms or electrons to unstable free radicals like superoxide anions, hydroxyl radicals, and peroxyl radicals. This stabilizes the radicals and terminates chain reactions that lead to cellular damage [17].

(b) Inhibition of lipid peroxidation

Moringa seed extracts prevent oxidative lipid degradation by shielding polyunsaturated fatty acids in cell membranes from oxidative degradation. This prevents membrane destabilization and preserves cellular integrity, particularly in oxidative stress-related conditions [5].

(c) Metal chelation and redox regulation

Flavonoids and tannins are chelators of transition metals like Fe^{2+}/Fe^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, Pb^{2+}, Cd^{2+}, Cr^{3+}, $Hg^{2+}$and Cu^{2+}, which facilitate the formation of highly reactive hydroxyl radicals through Fenton reactions. By reducing metal availability, these compounds limit oxidative reactions and cellular damage [2] [47].

(d) Modulation of endogenous antioxidant enzymes

Recent studies indicate that *Moringa* seed extracts enhance inherent antioxidant defense systems by catalyzing enzymes like glutathione peroxidase,superoxide dismutase (SOD), and catalase. This contributes to systemic oxidative balance and cellular protection [48].

The antioxidant activity of *Moringaoleifera* seeds is due to reduced risk of chronic diseases like as cancer, neurodegenerative disorders, diabetes, and cardiovascular diseases[4] [5] [10] [49] [51]. This supports their use in nutraceutical formulations and functional foods.

2.2.3 Anti-inflammatory activity

Moringaoleifera seed extracts exhibitanti-inflammatory effects mediated by multiple phytochemicals that regulate inflammatory pathways at molecular and cellular levels [2] [9].

(a) Inhibition of pro-inflammatory enzymes

Flavonoids and phenolic compounds inhibit lipoxygenase (LOX) and cyclooxygenase (COX) thereby reducing the synthesis of leukotrienes and prostaglandins, which are key mediators of inflammation [9] [11] [22].

(b) Suppression of cytokine production

Moringa seed extracts suppress the expression of pro-inflammatory cytokines like TNF-α, IL-1β, and IL-6. This modulation helps prevent severe inflammation and associated tissue damages [22].

(c) Regulation of nitric oxide pathways

The extracts of *Moringa oleifera* seedinhibit inducible nitric oxide synthase (iNOS), reducing excessive nitric oxide (NO) production, which is implicated in inflammatory and oxidative damage [25].

(d) Stabilization of cellular and lysosomal membranes

Tannins and saponins contribute to membrane stabilization, preventing the release of inflammatory mediators and enzymes from lysosomes[9] [11] [22].The anti-inflammatory activity of *moringa* seeds isbeneficial in managing inflammatory conditions like metabolic syndrome, arthritis, and inflammatory bowel diseases[9].

2.2.4Anticancer activity

The anticancer potential of *Moringaoleifera* seed extracts is increasingly supported by recent studies demonstrating their ability to target multiple pathways involved in cancer development. Recent studies by [5] showed strong anticancer effects of *Moringaoleifera* seed methanol extract against liver, breast, and colon cancer cell lines [5] [48]. The seed extract also showed antiproliferative effects against MCF-7 breast cancer cells [51]. In addition, sugar-rich seed fractions showed promising cytotoxicity in breast cancer models [49]. The anticancer effects of *Moringaoleifera*seed extract is highlighted below:

(a) Induction of apoptosis

Moringa seed phytochemicals activate intrinsic apoptotic pathways by regulating caspases and proteins like Bax, Bcl-2, leading to cell death in cancer cells [5].

(b) Cell cycle arrest

The extracts of *Moringa* seed interfere with cell cycle progression at the G1/S and G2/M checkpoints, thereby inhibiting uncontrolled proliferation of cancer cells [2].

© Anti-angiogenic effects
Moringa seed extracts inhibit angiogenesis by suppressing vascular endothelial growth factor (VEGF), limiting tumor growth and metastasis[2] [5].

(d) Reduction of stress-induced DNA damage
Moringa seed extracts scavenge free radicals, preventing mutations and DNA damage that could lead to carcinogenesis[2] [5].

(e) Selective cytotoxicity
Importantly, *moringa* seed extracts show selective toxicity toward cancer cells but not harming normal cells. This property makes *Moringa oleifera* seeds promising candidates for safe anticancer therapies [17].

2.2.5 Cardiovascular protective effects
Moringaoleifera seeds contribute significantly to cardiovascular health through lipid regulation, antioxidant effects, and vascular protection as highlighted below:
(a) Cholesterol-lowering effects
Saponins and phytosterols reduce intestinal absorption of cholesterol by binding bile acids, leading to decreased levels of LDL cholesterol and improved lipid profiles [2] [26].

(b) Prevention of atherosclerosis
Phenolic compounds prevent oxidation of LDL cholesterol, a major factor in plaque formation within blood vessels[9].

(c) Blood pressure regulation
Flavonoids enhance nitric oxide availability, promoting vasodilation and reducing blood pressure. This improves overall cardiovascular function [9].

(d) Anti-inflammatory effects on blood vessels
Moringa seed extracts reduce vascular inflammation, thereby protecting against endothelial dysfunction and atherosclerosis[5].

(e) Improvement of endothelial function
The extracts improve endothelial integrity and function, which is essential for maintaining vascular tone and preventing cardiovascular diseases[9].

2.3Mechanism of Actions of Bioactive Compounds in *Moringaoleifera* Seed Extracts
The antimicrobial potentials of *Moringaoleifera* seed extracts are mediated through multiple biochemical and molecular mechanisms. These mechanisms target essential microbial structures and functions, are often complementary, and involve different classes of phytochemicals acting synergistically resulting to the inhibition or killing of pathogens. Understanding these mechanisms is important as it provides a perspective insight on how *Moringaoleifera* seed extracts exhibit antimicrobial activity and support its prospective applications in the development of plant-derived antimicrobial agents. Below are the key mechanisms of action:
2.3.1 Membrane integrity compromise
Saponins and terpenoids interfere with microbial cell membranes by binding to membrane sterols and phospholipids. This interaction results to enhanced membrane permeability, resulting in leakage of essential intracellular components like proteins, nucleic acids, and ions. The loss of membrane integrity ultimately causes cell death [31] [39] [40] [41] [42] [43] [46] [47].

2.3.2 Inhibition of cell wall synthesis
Certain phytochemicals interfere with the synthesis of structural components of microbial cell walls, particularly peptidoglycan in bacteria. This makes the cell wall weak, resulting to the microorganism being susceptible to osmotic pressure and eventually, cell lysis. Gram-positive bacteria are more susceptibletothis mechanism[7] [21].

2.3.3 Protein denaturation and enzyme inhibition
Tannins, alkaloids, and phenolic compounds exert antimicrobial ability by binding to microbial proteins and enzymes. This binding leads to protein denaturation and enzyme inactivation, disrupting essential metabolic processes required for microbial survival. As a result, microbial growth and cell duplication are inhibited [10] [39].

2.3.4 Interference with nucleic acid synthesis
Alkaloids, phenolic compounds, and flavonoids can penetrate microbial cells and interact with nucleic acids. They inhibit enzymes such as DNA gyrase and topoisomerase, essential for DNA replication and transcription necessary for microbial replication and growth. This prevents microbial cell division and proliferation [31].

2.3.5 Induction of oxidative stress
Bioactive antioxidant compounds such as phenolics can promote the production of reactive oxygen species (ROS) within microbial cells. The accumulation of ROS leads to oxidative-induced cellular components compromise, including lipids, proteins, and DNA damages. This oxidative damage disrupts cellular function and contributes to microbial cell death [4].

2.3.6 Binding with essential nutrients
Some bioactive compounds in *Moringaoleifera* seeds chelate essential metal ions such as iron, calcium, and zinc thereby starving microorganism of the essential nutrients for growth, development and, other metabolic functions. This leads to cell starvation and death [6].

2.3.7 Inhibition of biofilm formation
Biofilms provide protection to microorganisms against antimicrobial agents. Certain bioactive compounds interfere with quorum sensing(bacterial communication systems), hence preventing biofilm formation, and decreasing virulence factor. Phytochemicals in *moringa* seeds inhibit biofilm formation by preventing microbial surface affirnity and disruption of established biofilms. This enhances the susceptibility of microorganisms to antimicrobial agents and reduces the persistence of infections [13] [21].
Comparative investigations have indicated that extracts from *Moringaoleifera* possess antimicrobial effects that, in some cases, are similar to those of certain standard antibiotics, highlighting their potential as alternative therapeutic agents. Nevertheless, differences in extraction techniques, microbial strains tested, and experimental setups make it essential to conduct more standardized and controlled studies to confirm these observations.

Figure 2: Pharmacological activities of Moringaoleifera seeds

2.4 Influence of SolventPolarity on Yield and Bioactivity

Antimicrobial potency is influenced by extraction method, solvent, concentration of phytochemical, microbial strains and resistance profile, and environmental factors like pH temperature, and pressure among others [16]. Solvents likewater, ethanol, methanol, and ethyl acetate represent solvents spanning a broad polarity range and influence both yield and potency of the antimicrobial phytochemicals present. Previous studies revealed that extraction using these solvents reveals a wide spectrum of phytochemicals like alkaloids, tannins saponins, phenolic compounds, flavonoids, steroids (phytosterols), terpenoids, and glycosides [18] [25] [38]. These compounds differ in polarity and solubility, which explains the variation in biological activity across different solvent extract. Organic solvents such as ethanol and methanol generally produce extracts with higher antimicrobial activity because they efficiently dissolve phenolic and flavonoid compounds ([18]. Aqueous extract, although safer and more accessible, tend to show comparatively lower activity due to limited extraction of non-polar bioactive constituents [38]. The differential solubility of phytochemicals in these solvents leads to qualitative and quantitative differences in the extract composition, which may significantly influence antimicrobial potential.

Water, as a polar solvent is the most traditional extraction solvent which is widely used in ethnomedicine. It predominantly extracts hydrophilic compounds, including glycosides, flavonoid glycosides, tannins, some phenolic acids, water-soluble proteins, and peptides[38].Aqueous extracts of *M.oleifera* seeds often contain antimicrobial proteins, including coagulant peptides that exhibit bacteriostatic properties. A research by [7] demonstrated that aqueous seeds extracts of *M.oleifera* inhibited *Escherichia coli* and *Staphylococcus aureus*, although activity was generally lower than alcoholic extracts. However, water has poor extraction of non-polar compounds (e.g., sterols, fatty acids), low stability of extracts during storage, and reduced diffusion capacity in agar assays. Despite these limitations, aqueous extracts are attractive because of safety, availability, cost-effectiveness, and compatibility with traditional applications.

Methanol and ethanol, both polar organic solvents, are widely used in phytochemical studies because of their ability to solubilize a wide spectrum of secondary metabolites like phenolics, flavonoids, terpenoids, tannins, alkaloids, and low molecular weight phytochemicals. Because many antimicrobial phytochemicals are phenolic in nature, methanolic extracts often demonstrate strong antimicrobial activity.

A research conducted by [35] showed thatmethanolic extract yielded higher content of total phenolic content, compared to aqueous extract. A similar research by[13] reported notable antimicrobial effect from methanolic seeds extract of*Moringa oleifera*. Methanol is however toxic and unsuitable fortherapeutic formulations without complete solvent removal,limiting its application mainly tolaboratory research.

Ethanol is widely regarded as an optimal solvent for antimicrobial phytochemical extraction because it dissolves both moderatelynon-polar and polar and compounds. The solvent extracts typically flavonoids, phenolic acids, saponins, alkaloids, and some lipophilic constituents. A research by [38] recorded that ethanolic extract of *M.oleiera* seeds exhibitedhnaced inhibitory activity againstGram-positive and Gram-negative bacteria, with higher inhibitionvalues than aqueousextract.In addition, ethanol has a broad-spectrum extraction capacity, lower toxicity compared to methanol and better penetration through microbial membranes. Ethanolic extracts frequently show stronger antimicrobial activities against *Staphylococcus aureus, Bacillus subtillis, Pseudomonas aeruginosa*and *Escherichia coli*.

Ethyl acetate, of intermediate polarity, often targets moderately polar compounds like certain flavonoidsaglycones, terpenoids, certain alkaloids, tannins, and lipophilic phenolics/phenolic esters. Because ethyl acetate excludes highly polar impurities, it often yields extracts with concentrated antimicrobial fractions. In comparative antimicrobial assays, ethyl acetate extracts have demonstrated superior potential against both Gram-positive and Gram-negative bacteria when compared with aqueous extracts. Ethyl acetate fractions of *M. oleifera* seeds produced larger inhibition zones that crudeaqueousextracts [38]. This is attributable to the selective extraction of bioactive fractions, reduced co-extraction of sugars and proteins. Gram-positive bacteria are however reported to be generally more vulnerable due to simpler cell wall structure. According to the report, Gram-negative bacteria however exhibited greater resistance because of their outer membrane barrier. It should be noted however that ethyl acetate may extract fewer hydrophilic bioactive peptides compared to water.

Table 2 compares antimicrobial activities against different solvents.

Table 2: Comparative antimicrobial activity across solvents

Solvent	Polarity	Phytochemical extracted	Relative Antimicrobial Activity
Water	High	Tannins, proteins, glycosides	Moderate
Ethanol	Moderately-High	Flavonoids, phenolics, saponins	High
Methanol	High	Phenolics, flavonoids, tannins	Very High (Lab studies)
Ethyl acetate	Moderate	Terpenoids, flavonoid aglycones	High to very high

3.0 Materials and Methods

3.1 Study area and sample collection

Fresh mature pods of *M. oleifera* were obtained from cultivated trees in the morning (when phytochemical fluctuations are lower) in Katsina-Ala, Benue State, Nigeria. Botanical identification was confirmed at the Department of Biology, College of Education, Katsina-Ala by a plant taxonomist.

3.2 Sample preparation

Seeds were removed from*M. oleifera* pods, washed, dried in a shade for 10 days, ground into fine powder using a sterile electrical laboratory grinder, and stored in airtight, light-proof vials at 20ºC until extraction.

3.3 Extraction procedure

One hundred grams of powdered seed were soaked separately in 500 mL distilled water, Methanol (analytical grade), ethanol (95%), and ethyl acetate. Cold maceration was performed for 72 hours while shaking intermittently. Extracts were filtered using Whatman No.1 filter paper, concentrated using a rotary evaporator (organic solvents) and water bath (aqueous extract). Extracts were stored at 4°C until analysis.

Figure 3: Moringaoleifera (a) Pods, (b) Seeds,(c) Powder, and (d) Extract

3.4 Determination of extraction yield

Percentage yield was calculatedas follows:

$$\text{Yield } (\%) = \frac{Weight\ of\ driedextract}{Weight\ of\ powdered\ sample} \text{ x } 100 \text{ .....(1)}$$

3.5 Phytochemical Analysis

3.5.1 Qualitative phytochemical analysis

Preliminary phytochemical evaluation of the extracts was carried out to detect major classes of bioactive compounds like alkaloids,tannins, flavonoids, phenolic compounds, terpenoids,saponins, glycosides responsible for antimicrobial activity using standard methods [52]. Each test was conducted using standardized pharmacognostic procedures under controlled laboratory conditions. All analyses were performed in triplicate to ensure reproducibility.

(i) Alkaloids (Meyer's Test)

Approximately 2 mL of extract solution was acidified using 1% HCl and filtered. Two drops of Mayer's reagent were added to the filtrate. Formation of creamy or pale-yellow precipitate indicated a positive test for alkaloids.

(ii) Flavonoids (Shinoda's Test)

To 2 mL of extract, small quantity of magnesium turnings was added, followed by concentrated HCl. Development of a pink, red, or orange coloration confirmed flavonoids.

(iii) Tannins (Ferric chloride Test)

$FeCl_3$ (5 %) was treated with 2 mL of the extract in a test tube. The appearance of a blue-black coloration showed positive test for hydrolysable or condensed tannins respectively.

(iv) Saponins (Frothing Test)

Frothing test was conducted by vigorously mixing 2 mL of extract with distilled waterin a test tube. Persistent foam lasting for more than 15 minutes suggested saponins.

(v) Terpenoids (Salkowski Test)

Salkowski test was applied by mixing 1 mL extract with two mL chloroform , $CHCl_3$followed by careful addition of 2 mL concentrated H_2SO_4. A reddish-brown interface indicated terpenoids.

(vi) Glycosides (Keller-Killiani Test)

Keller–Killiani test was carried out by mixing 1 mL extractwith 1 mL glacial acetic acid, three drops of $FeCl_3$, and one mL concentrated H_2SO_4. Brown ring at the interface after addition of ferric chloride and sulfuric acid indicated positive reaction for cardiac glycosodes.

(vii) Steroids (Liebermann-Burchard Test)

Two mL of the extract was treated with two mL ofethanoic anhydride, followed by two mL concentrated sulphuric acid, H_2SO_4 down the side in a test tube. Green or bluish-bluish color indicated a positive test.

(viii) Phenolics (Ferric chloride Test)

A volume of 2 mL of the extract was treated with three drops of 5% ferric chloride ($FeCl_3$) solution in a test tube. The appearance of a deep blue coloration indicated the presence of phenolic compounds.

3.5.2 Quantitative phytochemical determination

(i) Total phenolic content (TPC)

The total phenolic content was estimated using the Folin–Ciocalteu colorimetric method. Briefly, 0.5 mL of the extract (1 mg/mL) was combined with 2.5 mL of 10% Folin–Ciocalteu reagent. After 5 minutes, 2 mL of 7.5% sodium carbonate was added. The mixture was incubated at room temperature for 30 minutes, and absorbance was recorded at 765 nm using a UV–Visible spectrophotometer. A calibration curve was prepared using gallic acid (0–100 µg/mL). Results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g), calculated using:

$$TPC = \frac{C\ x\ V}{M} \text{.....(2)}$$

Where:

C = concentration from calibration curve (mg/mL), V = volume of extract (mL) and M = mass of extract (g). Results were expressed as mg gallic acid equivalent per gram (mg GAE/g).

(ii) Total flavonoid content (TFC)

Flavonoid content was determined using the aluminum chloride colorimetric assay. In this procedure, 0.5 mL of the extract was mixed with 1.5 mL methanol, followed by the addition of 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M potassium acetate. The mixture was incubated at room temperature for 30 minutes, after which absorbance was measured at 415 nm.

A standard curve was generated using quercetin (0–100 µg/mL), and results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g).

(iii) Tannin content

Tannin content was quantified using the Folin–Denis method. One milliliter of the extract was mixed with 5 mL of Folin–Denis reagent, followed by the addition of 10 mL sodium carbonate solution. The mixture was incubated for 30 minutes, and absorbance was measured at 700 nm. A tannic acid standard curve was used, and results were expressed as mg tannic acid equivalents per gram of extract (mg TAE/g).

(iv) Alkaloid content (Gravimetric method)

Five grams of the extract were dissolved in 10% acetic acid in ethanol and allowed to stand for 4 hours. The solution was then concentrated to one-quarter of its original volume. Alkaloids were precipitated by adding ammonium hydroxide dropwise. The resulting precipitate was filtered, dried at 60°C, and weighed. The percentage alkaloid content was calculated as:

Alkaloid (%) = (Weight of residue / Initial sample weight) × 100

$$\text{Alkaloid (\%)} = \frac{Weight\ of\ residue}{Initial\ sample\ weight} \text{x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

(v) Saponin content

Five grams of the sample were extracted with aqueous ethanol and heated at 55°C for 4 hours. The mixture was filtered, and the filtrate was concentrated before partitioning with diethyl ether. The aqueous layer was collected and evaporated to dryness. The remaining residue was weighed, and saponin content was expressed as a percentage:

$$\text{Saponin (\%)} = \frac{Weight\ of\ residue}{Initial\ sample\ weight} \text{x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

(vi) Terpenoid content (Vanillin-sulfuricacid method)

Two milliliters of the extract were dissolved in 95% ethanol to obtain a concentration of 1 mg/mL. From this, 0.5 mL was transferred into a test tube, followed by the addition of 1.0 mL of 5% vanillin solution in ethanol and 2.5 mL of concentrated sulfuric acid. The mixture was thoroughly mixed and incubated in a water bath at 60°C for 15 minutes, then cooled to room temperature. Absorbance was measured at 548 nm against a blank. Results were calculated from a calibration curve and expressed as mg terpenoid equivalents per gram of extract (mg TE/g).

$$TE = \frac{C\ x\ V}{M} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

Where:

C = concentration from calibration curve (mg/mL), V = volume of extract (mL) and M = mass of extract (g). Results were expressed as mg Terpenoid Equivalent per gram ofextract (mg TE/g).

(vii) Glycoside Determination (Anthrone method)

To 1 mL of the extract, 4 mL of Anthrone reagent was added. The mixture was heated in a boiling water bath for 10 minutes, then cooled. Absorbance was measured at 620 nm. A glucose calibration curve was used, and results were expressed as mg glycoside equivalents per gram of extract (mg GE/g).

3.6 Microorganisms Tested

The microbial strains used in this study included *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Candida albicans*. These clinical isolates were obtained from the microbiology laboratory of Rev. Fr. Moses Orshio Adasu University, Makurdi, Benue State, Nigeria. The organisms were maintained on appropriate culture media prior to use.

3.7 Antimicrobial Assay

The antimicrobial activity of the extracts was evaluated using the disk diffusion method, along with determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC). Extract concentrations of 25, 50, 75, and 100 mg/mL were tested against the isolates following established protocols without modification. Ciprofloxacin (0.5 mg/mL) and fluconazole (0.5 mg/mL) were used as positive controls for bacterial and fungal strains, respectively.

Bacterial isolates were cultured on Nutrient Agar and incubated at 37°C for 24 hours, while fungal isolates were grown on Potato Dextrose Agar at 25–30°C for 48 hours. Microbial suspensions were standardized to 0.5 McFarland turbidity, corresponding to approximately 1.5 × 10^8 CFU/mL for bacteria, to ensure uniformity in testing.

3.8 Minimum Inhibitory Concentration (MIC), Minimum Bactericidal/Fungicidal Concentration (MBC/MFC)

The MIC was determined using the broth dilution technique. Serial two-fold dilutions of the extract were prepared and inoculated with standardized microbial suspensions. After incubation under appropriate conditions, microbial growth was assessed.

For MBC/MFC determination, samples from tubes showing no visible growth during MIC testing were subcultured onto fresh agar plates and incubated. The lowest concentration that resulted in no microbial growth (indicating 99.9% killing) was recorded as the MBC or MFC.

This microbial evaluation provides insight into the antimicrobial potential of *Moringa oleifera* seed extracts and supports their possible application as natural antimicrobial agents.

3.9 Statistical Analysis

All experiments were conducted in triplicate to ensure reliability. Data obtained were analyzed using one-way analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$), followed by Tukey's Honestly Significant Difference (HSD) post hoc test. Results were presented as mean ± standard deviation (Mean ± SD). Statistical analyses were performed using SPSS version 21, and results were illustrated using tables and bar charts.

4.0 Results and Discussion

4.1 Results

4.1.1 Extraction yield

Table 3: Percentage yield of extract

S/No.	Solvent	Mean ± SD (%)
1.	Methanol	$18.73^{a} \pm 0.21$
2.	Ethanol	$16.93^{b} \pm 0.20$
3.	Water	$12.40^{c} \pm 0.26$
4.	Ethyl acetate	$10.27^{d} \pm 0.15$

N= 3, values expressed as Mean ± SD. Different alphabetical letters (superscripts) show significant difference p < 0.05.

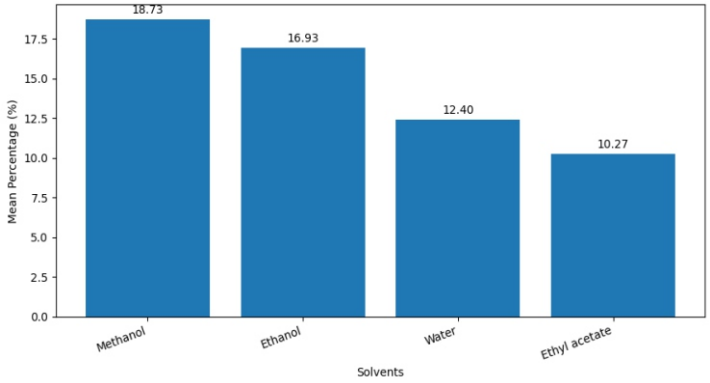

Figure 4: Percentage yield of extracts of Moringaoleifera seeds

4.1.2 Qualitative phytochemical test result

Table 4: Phytochemical analysis result

Secondary Metabolite	Test	Result
Alkaloids	Mayer's test	+
Flavonoids	Shinoda's test	+
Tannins	Ferric Chloride test	+
Saponins	Frothing test	+
Terpenoids	Salkwoski's test	+
Glycosides	Keller-Killiani's test	+
Steroids	LibbermannBurchard's test	+
Phenolics	Ferric Chloride test	+

Key: *+ = Presence of secondary metabolite*

4.1.3 Quantitative phytochemical distribution

Table 5: Quantitative phytochemical spectrum

Phytochemical	Water	Methanol	Ethanol	Ethyl acetate
Alkaloids	+	++	++	+
Flavonoids	+	++	++	+
Tannins	+	++	+	+
Saponins	++	++	+	-
Terpenoids	_	++	++	+
Phenolics	+	++	++	+
Steroids	-	+	+	+
Glycosoides	+	++	+	+

Key: *++ = Strongly present; + = Present; - = Absent*

4.1.4 Quantitative phytochemical content

Table 6: Quantitative Phytochemical Composition of M.oleifera seeds extracts

Solvent	TPC (mgGAE/g)	TFC (mg QE/g)	Tannins (mg TAE/g)
Methanol	$85.40^{a} \pm 2.10$	$62.32^{a} \pm 1.80$	$41.20^{a} \pm 1.50$
Ethanol	$79.61^{b} \pm 1.90$	$58.70_{b} \pm 1.61$	$38.40^{b} \pm 1.30$
Water	$55.82^{d} \pm 1.70$	$39.40^{d} \pm 1.21$	$6.31^{d} \pm 1.40$
Ethyl acetate	$60.21^{c} \pm 4.40$	$42.40^{c} \pm 2.00$	$29.70^{c} \pm 1.812$

N= 3, values expressed as Mean ± SD. Values in the same column with different superscripts have significant correlation at $p < 0.05$.

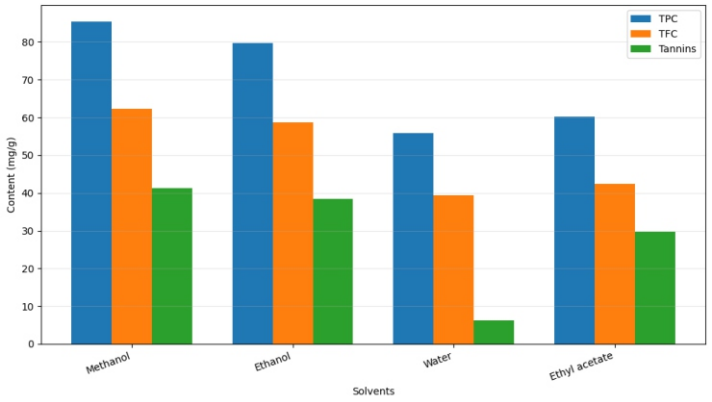

Figure 5: Quantitative Phytochemical Composition of M. oleifera seeds extracts

4.1.5 Antimicrobial activity (InhibitionZone, mm)

Table 7: Average inhibition (mm) zone of methanol extract

Test Organism	Concentration 100	(mg/mL) 75	50	25	control
S. aureus	$20.12^{b} \pm 0.9$	$18.10^{c} \pm 1.0$	$15.10^{d} \pm 0.9$	$11.10^{e} \pm 0.9$	$28.15^{a} \pm 2.0$
E. coli	$18.00^{b} \pm 0.9$	$16.09^{c} \pm 0.9$	$14.91^{d} \pm 0.9$	$10.10^{e} \pm 0.8$	$31.10^{a} \pm 2.0$
P. aeruginosa	$17.12^{b} \pm 1.0$	$15.14^{c} \pm 1.0$	$13.12^{d} \pm 0.8$	$9.11^{e} \pm 0.9$	$26.00^{a} \pm 2.5$
K. pneumonia	$15.11^{b} \pm 0.9$	$13.15^{c} \pm 0.9$	$11.10^{d} \pm 0.9$	$8.10^{e} \pm 0.8$	$28.10^{a} \pm 2.0$
C. albicans	$18.00^{b} \pm 0.8$	$16.10^{c} \pm 0.9$	$14.10^{d} \pm 0.9$	$10.00^{e} \pm 0.9$	$31.00^{a} \pm 2.0$

N= 5, values expressed as Mean ± SD. Values in the same row with different superscripts are significantlydifferent $p < 0.05$.

Table 8: Average inhibition (mm) zone of ethanol extract

Test Organism	Concentration 100	(mg/mL) 75	50	25	control
S. aureus	$18.10^{b} \pm 0.8$	$16.00^{c} \pm 1.1$	$14.11^{d} \pm 0.8$	$10.10^{e} \pm 1.0$	$28.15^{a} \pm 2.0$
E. coli	$16.10^{b} \pm 0.8$	$15.10^{c} \pm 1.0$	$12.82^{d} \pm 1.0$	$9.14^{e} \pm 1.0$	$31.10^{a} \pm 2.0$
P. aeruginosa	$15.11^{b} \pm 0.9$	$13.15^{c} \pm 0.9$	$11.10^{d} \pm 0.9$	$8.10^{e} \pm 0.8$	$26.00^{a} \pm 2.5$
K. pneumonia	$17.10^{b} \pm 0.8$	$15.09^{c} \pm 1.0$	$13.12^{d} \pm 0.8$	$9.11^{e} \pm 0.4$	$28.10^{a} \pm 2.0$
C. albicans	$16.10^{b} \pm 0.9$	$14.14^{c} \pm 0.9$	$12.00^{d} \pm 0.8$	$8.12^{e} \pm 0.8$	$31.00^{a} \pm 2.0$

N= 5, values expressed as Mean ± SD. Values in the same row with different superscripts are significantlydifferent $p < 0.05$.

Table 9: Average inhibition zone (mm) of water extract

Test Organism	Concentration 100	(mg/mL) 75	50	25	Control
S. aureus	$12.00^{b} \pm 0.9$	$10.12^{c} \pm 1.1$	$8.12^{d} \pm 1.0$	$6.12^{e} \pm 1.0$	$28.15^{a} \pm 2.0$
E. coli	$10.10^{b} \pm 0.9$	$9.11^{c} \pm 0.9$	$7.15^{d} \pm 0.9$	$5.10^{e} \pm 0.8$	$31.10^{a} \pm 2.0$
P. aeruginosa	$9.10^{b} \pm 0.9$	$8.11^{c} \pm 1.0$	$6.12^{d} \pm 0.9$	$4.13^{e} \pm 0.7$	$26.00^{a} \pm 2.5$
K. pneumonia	$11.00^{b} \pm 0.9$	$9.11^{c} \pm 0.8$	$7.12^{d} \pm 0.7$	$5.10^{e} \pm 0.7$	$28.10^{a} \pm 2.0$
C. albicans	$10.10^{b} \pm 0.9$	$9.10^{c} \pm 0.8$	$7.11^{d} \pm 0.8$	$4.00^{e} \pm 0.7$	$31.00^{a} \pm 2.0$

N= 5, values expressed as Mean ± SD. Values in the same row with different superscripts are significantlydifferent $p < 0.05$.

Table 10: Average inhibition zone (mm) of ethyl acetate extract

Test Organism	Concentration 100	(mg/mL) 75	50	25	Control
S. aureus	$15.14^{b} \pm 0.9$	$13.00^{c} \pm 1.0$	$11.10^{d} \pm 0.9$	$8.00^{e} \pm 0.9$	$28.15^{a} \pm 2.0$
E. coli	$14.11^{b} \pm 0.9$	$12.07^{c} \pm 0.8$	$10.11^{d} \pm 0.8$	$7.00^{e} \pm 0.9$	$31.10^{a} \pm 2.0$
P.aeruginosa	$13.00^{b} \pm 0.8$	$11.10^{c} \pm 0.9$	$9.13^{d} \pm 1.0$	$6.12^{e} \pm 0.9$	$26.00^{a} \pm 2.5$
K. pneumonia	$14.12^{b} \pm 0.9$	$12.00^{c} \pm 0.9$	$10.14^{d} \pm 0.9$	$7.14^{e} \pm 0.8$	$28.10^{a} \pm 2.0$
C. albicans	$13.00^{b} \pm 0.8$	$9.13^{c} \pm 0.8$	$7.12^{d} \pm 0.9$	$5.13^{e} \pm 0.9$	$31.00^{a} \pm 2.0$

N= 5, values expressed as Mean ± SD. Values in the same row with different superscripts are significantly different p < 0.05.

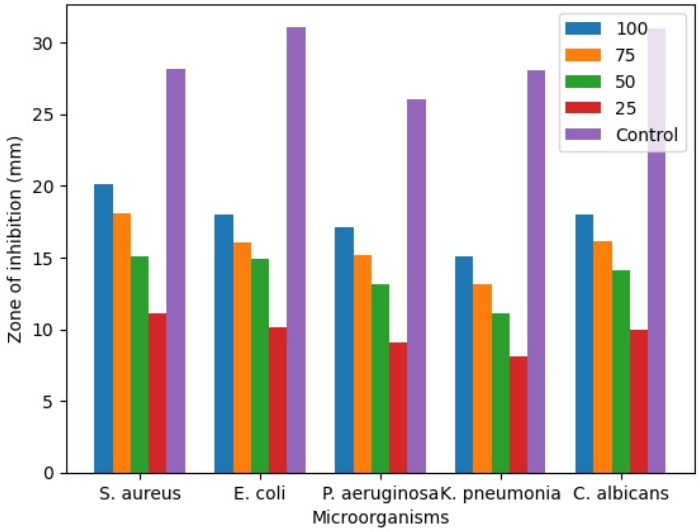

Figure 6: Antimicrobial activity of methanol extract

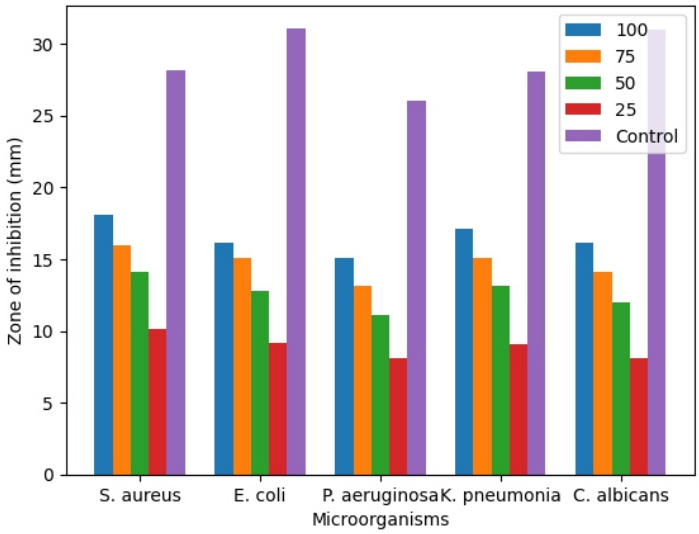

Figure 7: Antimicrobial activity of ethanol extract

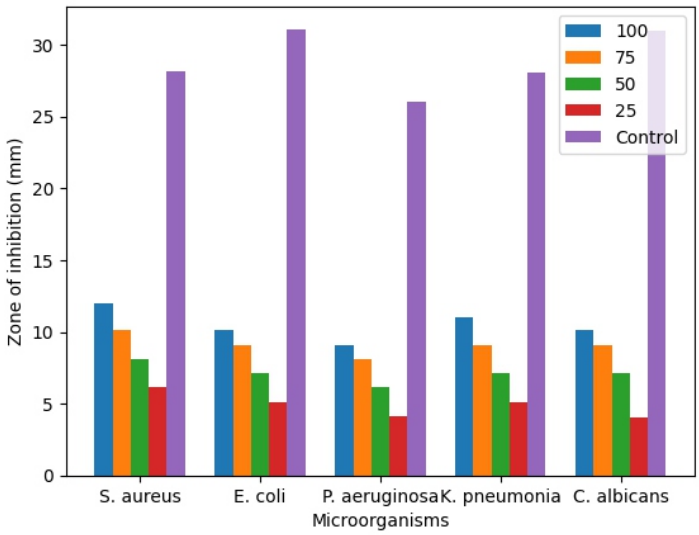

Figure 8: Antimicrobial activity of water extract

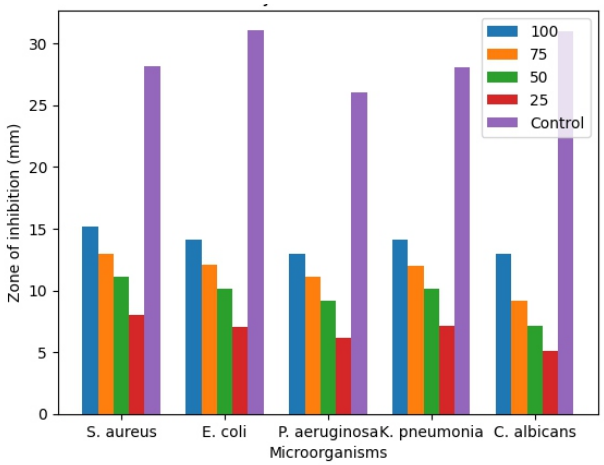

Figure 9: Antimicrobial activity of ethyl acetate extract

4.1.5 Minimum inhibitory concentration, MIC

Table 8: MIC Test Result

Solvent	Test Organism	MIC (mg/mL)	MBC/MFC (mg/mL)
Methanol	*Staphylococcus aureus*	12.5	25.0
	Escherichia coli	12.5	25.0
	Pseudomonas aeruginosa	12.5	25.0
	Klebsiella pneumoniae	12.5	25.0
	Candida albicans	12.5	25.0
Ethanol	*Staphylococcus aureus*	16.0	32.0
	Escherichia coli	20.0	40.0
	Pseudomonas aeruginosa	19.0	36.0
	Klebsiella pneumoniae	14.0	28.0
	Candida albicans	18.0	32.0
Water	*Staphylococcus aureus*	17.0	34.0
	Escherichia coli	25.0	50.0
	Pseudomonas aeruginosa	20.0	40.0
	Klebsiella pneumoniae	12.0	25.0
	Candida albicans	20.0	40.0
Ethyl acetate	*Staphylococcus aureus*	18.0	32.0
	Escherichia coli	25.0	50.0
	Pseudomonas aeruginosa	34.0	68.0
	Klebsiella pneumoniae	25.0	50.0
	Candida albicans	25.0	50.0

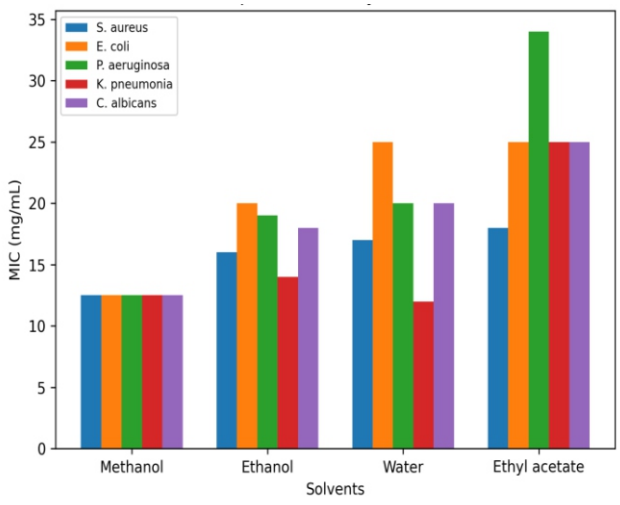

Figure 10: MIC of Moringaoleifera seed extracts

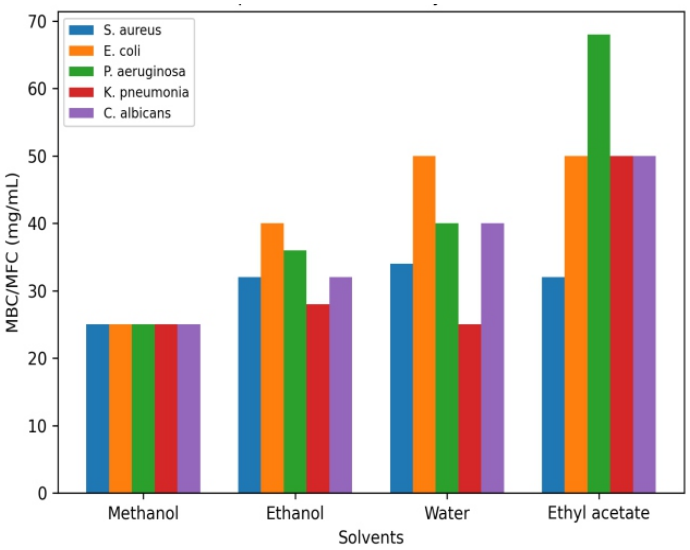

Figure 11: MIC of Moringaoleifera seed extract vs MBC across microorganisms

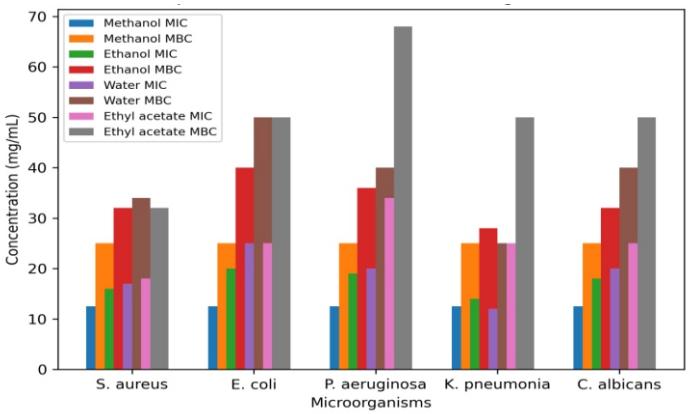

Figure 12: MIC/MBC by solvent

4.2 Discussion

The extraction yield result (Table 3) revealed that methanol produced the highest extraction yield (18.73 ± 0.21), followed by ethanol (16.93± 0.20), water (12.40± 0.26), while ethyl acetate yielded the least (10.27± 0.15). The result indicates that significant extraction yield variation exists among solvents.This variation yield reflects the polarity of the solvents and the chemical composition of the compounds in the seeds. The research finding is in agreement with that of [1] [7], [10], [12], [28], [38], and [50].Therefore, the selection of the solvent should be guided by the target compounds of interest as well as safety and application conditions.

The qualitative phytochemical screening (Table 4) revealed that *Moringaoleifera* seed extracts contain a wide range of phytochemicals, including steroidsalkaloids, glycosides, flavonoids, tannins, phenolics, saponins, terpenoids. The universal presence of these metabolites suggests that the seeds are chemically rich and may possess multiple biological activities. These category of compounds are widely responsible for antimicrobial, antioxidant, and therapeutic properties,which likely underpin the biological effects observed in subsequent analyses. Studies have shown that methanol and other polar organic solvents are particularly effective in extracting antimicrobial constituents from *M. oleifera* and other medicinal plants [18] [24]. The result correlated well with that of [38].

The comparative phytochemical distribution (Table 5) demonstrates clear solvent-dependent variations in extraction efficiency. Methanol exhibited the broadest and most intense phytochemical profile, indicating its superior capacity to solubilize both polar and moderately non-polar constituents. Ethanol showed a similar but slightly reduced extraction capacity, while ethyl acetate selectively extracted fewer compounds, particularly lacking saponins. Water extract displayed the least diverse phytochemical composition, reflecting its limitation in dissolving less polar bioactive substances as reported by similar researches like [14] [24] and [38]. This pattern indicates the strong influence of solvent polarity on phytochemical recovery.

Quantitative analysis (Tables 6) further confirmed these trends, with methanol extract recording the highest TPC, TFC, and tannin levels, followed by ethanol, water, and ethyl acetate. The statistically significant differences among the solvents indicate that methanol is most effective in extracting antioxidant-related compounds. A similar research by [38] indicated a similar trend. The relatively high values observed in methanol extracts suggest its higher polarity. However, ethanol is more suitableand a safer alternative solvent for extraction, especially in food, medical, and pharmaceutical applications.

The antimicrobial efficacy of the extracts (Tables 7–10) showed a concentration-dependent increase in inhibition zones across all tested organisms. Methanol extract (Table 6) consistently exhibited the highest antibacterial and antifungal activity, which correlates with its richer phytochemical composition. Ethanol extract (Table 7) demonstrated moderate activity, whereas ethyl acetate (Table 9) showed lower but still appreciable inhibition. Water extract (Table 8) produced the smallest zones of inhibition, indicating weaker antimicrobial potency. Previous researches have similarly documented lower antimicrobial effects in aqueous extracts compared to organic solvent extracts, emphasizing the influence of solvent selection in phytochemical extraction[25].

The minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) results (Table 10) confirm the antimicrobial results. Methanol extract demonstrated the lowest MIC and MBC/MFC values across all organisms, indicating the highest antimicrobial potency. The ethanol extract also demonstrated substantial antimicrobial potential, although slightly less than that of methanol. The observation is consistent with recent reports indicating that ethanol is capable of extracting both polar and moderately non-polar compounds, resulting in rich phytochemical reservoir with significant antimicrobial potential [16]. The comparable activity of methanol and ethanol extracts suggests that both solvents are suitable for recovering bioactive compounds, although methanol may offer slightly higher extraction efficiency for certain phytochemicals.

In contrast, the ethyl acetate extract exhibited moderate antimicrobial activity. This can be explained by its intermediate polarity, which favors the extraction of semi-polar compounds such as certain terpenoids and phenolic esters. While these compounds contribute to antimicrobial activity, their concentration and diversity may be lower compared to those extracted by more polar solvents [24]. Nevertheless, ethyl acetate extracts have been reported to contain important antimicrobial constituents, supporting their moderate effectiveness observed in this study.

The aqueous extract revealed the lowest antimicrobial efficacy among all solvents used.

This decreased efficacy may be due to the limited ability of water to dissolve non-polar and moderately polar bioactive compounds. Although water efficiently extracts hydrophilic substances like sugars and some glycosides, many potent antimicrobial phytochemicals, particularly phenolics and flavonoids are more soluble in organic solvents. Previous studies have similarly documented lower antimicrobial activity in aqueous extracts compared to organic solvent extracts, emphasizing the significance of solvent selection in phytochemical extraction [14].

Among the test organisms, *Staphylococcus aureus* and *Candida albicans* were generally more vulnerable, while *Pseudomonas aeruginosa* exhibited reduced sensitivity, likely due to its inherent resistance mechanisms. The standard controls showed significantly higher inhibition, confirming the validity of the result.

Furthermore, observed variation in susceptibility between Gram-positive and Gram-negative bacteria is consistent with established microbiological principles.Gram-positive bacteria(*Staphylococcus aureus*) was more susceptible to the extracts than Gram-negative bacteria (*Klebsiellapneumoniae* and *Pseudomonas aeruginosa*). This difference can be attributed to structural variations in their cell walls. Gram-positive bacteria possess a relatively simple peptidoglycan layer, which is more accessible to bioactive compounds, whereas Gram-negative bacteria have an additional outer membrane that acts as a permeability barrier, reducing the penetration of antimicrobial agents. Similar observations have been reported in recent studies on *M. oleifera*, where Gram-positive organisms exhibited higher sensitivity to plant extracts as confirmed by other researches [14] [24].

The antifungal property observed against *Candida albicans* further highlights broad-spectrum antimicrobial potential of *M. oleifera* seed extracts. This activity may be associated with the presence of phenolics and isothiocyanates, which have been reported to interfere with fungal cell membranes and inhibition of essential metabolic pathways. Additionally, bioactive compounds identified in *M. oleifera*, such as 4-hydroxybenzaldehyde and related phenolics, have been linked to antimicrobial effects in previous studies [24].

The antimicrobial properties of *Moringaoleifera* extracts are largely linked to the various bioactive compounds such as flavonoids, alkaloids, tannins, phenolics, and saponins. These phytochemicals exert their effects through several complementary mechanisms against microbial organisms. One major mechanism involves disruption of the microbial cell membrane. Compounds such as saponins and phenolics can alter membrane integrity, increasing permeability and causing leakage of intracellular materials, which ultimately leads to cell death[6] [17].

Another mode of action is the inhibition of key enzymatic processes. Flavonoids and alkaloids can interfere with enzymes required for nucleic acid synthesis, energy metabolism, and cell wall formation, suppressing microbial growth, development, and replication[21]. Tannins contribute by binding to and precipitating microbial proteins, including enzymes and structural components, which compromises cellular function and stability[5] [19]. Additionally, phenolic compounds may induce oxidative-induced damage within microbial cells via generating reactive oxygen species (ROS). These reactive molecules can damage essential biomolecules like DNA, proteins, and lipids as reported by [54].

Some phytochemicals are also believed to inhibit microbial efflux pumps, allowing antimicrobial substances to accumulate within the cell and enhancing their effectiveness. Collectively, these combined and often synergistic actionsaccount for the enhanced antimicrobial effects in *Moringaoleifera* extracts. The multifaceted nature of these interactions may also reduce the propability of resistance development compared to single-target conventional antimicrobial agents.

In summary, the findings of this study reinforce the critical role of solvent polarity in determining both the phytochemical chemistry and antimicrobial efficacy of plant extracts. Polar organic solvents, particularly methanol and ethanol, were more efficient in extracting bioactive compounds responsible for antimicrobial activity. Methanol proved to be the most effective solvent, although ethanol presents a viable and safer alternative for practical applications. The strong correlation between phytochemical richness and antimicrobial performance underscores the importance of optimizing extraction methods in development of plant-based antimicrobial agents.

4.3 Limitations of the Study

Although the study produced encouraging results, certain limitations should be acknowledged as they may influence how broadly the findings can be applied.

First, only a small number of microbial strains were examined. Including a wider range of clinically important bacteria and fungi would provide a more comprehensive determination of antimicrobial activity.

Second, the extraction methods were restricted to aqueous, methanol, ethanol, and ethyl acetate techniques. Alternative approaches like supercritical fluid extraction or ultrasonic-assisted extraction may reveal different phytochemical compositions and improve antimicrobial efficacy.

Another limitation is the absence of *in vivo* experiments. The study relied solely on *in vitro* assays, which may not fully reflect the behavior, safety, or effectiveness of the extracts in living organisms.

Furthermore, the study did not explore possible synergistic interactions between *Moringaoleifera*extracts and standard antibiotics. Such investigations could reveal opportunities for combination therapies.

Finally, while total phytochemical content was measured, individual compounds were not isolated or identified. This limits the ability to determine which specific constituents are primarily responsible for the observed antimicrobial effects.

Addressing these issues in future work would improve the robustness and applicability of the findings.

4.4 Implications for Medicine and Pharmacology

Results of this research emphasize the potentials of *Moringaoleifera* as a source of natural antimicrobial agents. The diverse phytochemicals such as flavonoids, phenolics, alkaloids, saponins, and tannins supports its relevance in therapeutic applications. Its antimicrobial activity against both bacterial and fungal pathogens suggests it could be used either as an alternative or as an adjunct to conventional antimicrobial drugs.

In medical practice, *Moringaoleifera* extracts may be useful in treating microbial infections, particularly those involving antibiotic-resistant microorganisms. Its broad-spectrum effects also shows potential applications in topical preparations, wound care, and pharmaceutical preservation.

From the pharmacological perspective, the identified phytochemicals provide promising candidates for drug development. Further research could focus on isolating and characterizing these compounds as lead molecules for new antimicrobial agents. Additionally, combining *Moringaoleifera*extracts with existing antibiotics may improve treatment efficacy and help mitigate resistance.

Overall, these findings support the inclusion of *Moringaoleifera* in pharmaceutical research, nutraceutical development, and traditional medicine as a sustainable and effective antimicrobial resource.

5.0 Conclusion

The antimicrobial effects of *Moringaoleifera* seed extracts is strongly solvent-dependent. Methanol extract demonstrated superior phytochemical recoveryand antimicrobial activity, followed by ethanol extract. However, methanol's toxicity limits pharmaceutical applications. Ethanol balanced polarity enabled extraction of broad-spectrum phytochemicals. Its antimicrobial performance was comparable to methanol, making it more suitable for therapeutic use.Ethyl acetate yielded selective concentration of potent semi-polar bioactive compounds, particularly terpenoids with low antimicrobial activity compared to methanol and ethonal. Water extracted hydrophilic tannins and saponins but showed lower antimicrobial potency. Limited membrane permeability of water-soluble compounds may explain reduced activity but remain relevant for traditional applications. Methanol emerges as the most practical solvent for pharmaceutical development due to its high efficacy and safety profile.

The findings align with recent research indicating that alcoholic and semi-polar extracts of *Moringaoleifera* seeds possess enhanced antimicrobial activity due to higher phenolic and flavonoid concentrations.

Future research should focus on compound isolation, synergy evaluation, toxicity profiling, and formulation studies.

5.1 Recommendations for Future Research

Based on the outcomes and limitations of this study, several directions are suggested for further investigations:

A broader range of isolates, including multidrug-resistant , should be tested to better assess antimicrobial efficacy.

Advanced extraction techniques like supercritical fluid, microwave-assisted, or ultrasonic-assisted methods should be explored to optimize the yield and diversity of active compounds.

Efforts should be made to isolate, purify, and structurally characterize individual bioactive constituents attributed to antimicrobial activity.

In vivo antimicrobial assays are necessary to evaluate the safety, efficacy, and pharmacokinetics of the extracts in biological systems.

Studies on combined interactions between *Moringaoleifera* extracts and conventional antibiotics should be conducted to improve therapeutic outcomes.

Further research should investigate the detailed molecular mechanisms responsible for antimicrobial effects of the plant's phytochemicals.

Implementing these recommendations will provide stronger scientific evidence for the use of *Moringaoleifera* as a dependable natural antimicrobial agent.

Acknowledgement

The authors highly acknowledge the staff of the Departments of Chemistry, Biological Sciences College of Education, Katsina-Ala, and Rev. Fr. Moses Orshio Universirt, Makurdi, Benue State, for the facilities provided and the assistance for the successful conduct of this research.

Contributions

This research was conceptualized and drafted by Dr. Terngu PaulUgosor. Dr. Apeyuan David Kparev-Wua and Naku Julius Uko prepared the tables, figures, and charts. All the authors reviewed and edited the original manuscript before submission for publication.

Ethics Declaration

Ethic Approval

No ethical approval was required forthis publication.

Consent for Publication

Consentfor publication is not applicable as the research does not include details, images,or videos relating to human participants, personaldata, or case studies requiring consent for publication.

Competing Interests

Authors have declared that there is no competing interests.

Declaration of Generative AI and AI-Assisted Technologies

In the course of preparation of this work, the authors used ChatGPT to paraphrase the original text for improved grammatical clarity. The authors thereafter, carefully revealed and edited the content and take full responsibility for the publication of the content.

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