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PHYTOCHEMICAL SCREENING AND MICROBIAL ASSESSMENT OF *Moringa oleifera* SEED CRUDE OIL EXTRACT

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ABSTRACT
The present inherent ability of an organism to human pathogenic infections compelled the need to explore new substances with antimicrobial properties from plant-based materials. *Moringa oleifera* seed extract was evaluated for antimicrobial effects against the selected bacteria strains - *Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* and selected fungi - *Rhizopus stolonifera* and *Candida albicans*. The zone of inhibition test revealed samples inhibited microbial growth to various extents with greater sensitivity against fungal as viewed in the bacterial strains. Phytochemical screening of analyzed samples revealed the presence of some metabolites (secondary) such as Alkaloids, saponins, flavonoids, Anthocyanin and betacyanin, Quinones, tannins, Terpenoids and Acids. Gas-chromatography-mass spectrometry (GCMS) analysis identified about 33 chemical components.
Keywords: Antimicrobial, Antifungal, *Moringa oleifera,* Phytochemical, Soxhlet Apparatus
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INTRODUCTION

Moring oleifera (*M. oleifera*) is grown world-wide, especially in Asia and Africa.[1, 2] Moringa is a native to Africa, Southeast Asia, Arabia, Caribbean islands, the Pacific and South America.[3, 4] Other countries where it is well-known include Philippines, Nigeria, Malaysia, Bangladesh, Pakistan and Kenya.[5,6] *Moringaceae* is of the oilseed trees family, categorized as a genus single tree with almost fourteen species. These species are not limited to *M. Oleifera*. However, also *M. drouhardii, M. Cocanensis, M. Stenopetala, M. Peregina and M. Pterygosperma.*[7] Of the fourteen known species, *M. Oleifera* stands out as the most widely known and utilized.[8] It is planted for its innumerable functional properties as it gives various nutritional and medicinal resources.[6,9,10]

Apart from the detrimental effects of repellent and synthetic drugs on man, it has been discovered that they are no longer effective and efficient due to adaptation of these microorganisms, different antimicrobial agent has been discovered in alleviating the global burden of pathogenic agents of diseases in the environment.[11, 12]

The active compounds in most plants and their extract have given them the ability to be used for several medicinal purposes. These active compounds/substances are known as phytochemicals which have the attribute of exerting a distinctive physiological influence on specific organisms.[13]

Several works has been done and reported by researchers on Phytochemicals in plant extract and their detrimental activities on some microorganisms and larva of some insects.[14] Phytochemicals can be grouped into two major parts; primary (chlorophyll and Amino acids) and secondary metabolites (Alkaloids, flavonoids, Tannins, Saponins etc).[14-16] Some of these phytochemicals have been discovered to possess antimicrobial, insecticidal, antioxidant activities, anticonstipative and antispasmodial properties.[17, 18]

M. oleifera seed is popularly known as Ben oil which is rich in some acids such as; oleic, behenic, steric and palmitic acids.[19,20] Noted the seed to consist of about 70% oleic acid, which made it suitable for frying.[21] It can also be used as lubricants to produce air care products, perfume, and other edible functions. It has similar composition of fatty acids as the olive oil[21] apart from the presence of linoleic

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acid.[22] Oil extract of *M. oleifera* seed is a good feedstock for biodiesel production.[23,24] The most notable characteristic of biodiesel produced from the oil extract of *M. oleifera* seed is the high value of cetane number, which is above 60 as reported.[25, 26-30] Have also reported the effect of several crude and isolated oil from various plants parts, such as *Eucalyptus spp., Cymbopogon spp*., *Menthra spicata, Hyptis saveolens* and *Ocimum spp*., against mosquitoes. *Cymbopogon* (Lemongrass) and *Hyptis saveolens* (*scent leave*) *are* used locally to repel mosquitoes in rural regions. In this work, plant-based materials were researched as alternatives to synthetic antimicrobial substances.

EXPERIMENTAL

Collection of Samples

Dry seed of *M. oleifera* was purchased from the open market in Ota, a town in Ogun State, Nigeria.

Seed preparation and Extraction

Seed preparation was carried out and extracted [31-34] using reported methods.

Phytochemical Screening

Methods[12, 17, 35, 36] were employed for qualitative phytochemical screening of seed crude oil extracts.

Bacterial Strains

Clinical isolates of *B. subtilis, S. aureus, E. coli, K. pneumonia, P. aeruginosa, C. albican* and *Rhizopus* were obtained from Biological Sciences Department, Covenant University, Ota, Ogun State.

Antimicrobial Analysis

Testing for the Antimicrobial property of crude Oil extract

Methods [33,37, 38] were used for the test of antimicrobial property of crude oil extract.

Minimum Inhibitory Concentration Determining (MIC)

Modified methods[37, 38] were employed for MIC determination.

Determining the Minimum Bactericidal Concentration (MBC)

Modified methods[37, 38] were used for MIC determination.

RESULTS AND DISCUSSION

Phytochemical Screening

Table-1 shows why *Moringa oleifera* seed is used in traditional medicine.

Table-1: Phytochemical Analysis of *M. Oleifera* Seed Oil Extract

Phytochemical Test	Crude Oil Extract
Alkaloid	+
betacyanin and Anthocyanin	+
Glycosides	–
Cardiac glycosides	–
Terpenoids	+
Phenols	–
Acids	+
Carbohydrate	-
Saponins test	+
Flavonoid	+
Tannins	+

Key: (+) = Present (-) = Absent

Antimicrobial Test Result

The Phytochemicals identified in Table-1 revealed why *Moringa oleifera* seed oil extract could be used as a good substitute for synthetic microbial drugs and cream.

Table-2: Antimicrobial Property of Crude Oil Extract

Organisms	Zone of Inhibition(mm)		
	Extract	Control Gentamicin	Commercial Extract
B. subtilis	5	15	4
S. aureus	5	12	3
E. coli	4	5	3
K. pneumoniae	4	10	3
P. aeruginosa	5	11	4
		Control Nystatin	
C. albicans	40	30	22
R. stolonifera	15	33	10

MIC and MBC Analysis

The MIC and MBC results show that the seed oil extract has antimicrobial properties against some selected microorganisms which can be used for the treatment of infectious diseases.

Table-3: MIC Determination

Organisms	The concentration of *M. Oleifera* Seed Crude Oil Extract (mg/ml)				
	100.00	50.00	25.00	12.50	6.25
B. subtilis	-	+	+	+	+
E. coli	_	_	-	+	+
S. aureus	_	_	+	+	+
K. pneumoniae	_	_	_	+	+
P. aeruginosa	_	+	+	+	+
C. albicans	_	_	_	+	+
R. stolonifera	_	_	_	+	+

Key:(+) = Growth was noticed (-) = Growth was absent (inhibited)

Table-4: MBC Determination of *M. oleifera* Seed Extract

Organisms	*M. Oleifera* crude Seed Oil extract Concentration(mg/ml)				
	100.00	50.00	25.00	12.50	6.25
B. subtilis	+	+	+	+	+
S. aureus	_	_	+	+	+
E. coli	_	_	+	+	+
K. pneumoniae	_	+	+	+	+
P. aeruginosa	_	+	+	+	+
C. albicans	_	+	+	+	+
R. stolonifera	_	+	_	+	+

Key:(+) = growth observed (-) = Growth inhibited

Gas Chromatography-Mass Spectrometry (GCMS)

The GCMS analysis carried out shows about 33 compounds of which Oleic, cis-13-Octadecenoic and cis-Vaccenic acid had appreciable composition. These 3 compounds can be used as pharmaceutical solvent which serves different purposes in medicine.

Table-5: *M. oleifera* Seed Oil Extracts Chemical Composition of using GCMS

Pk #	Retention Time	% Area	Composition
1	20.710	2.37	n-Hexadecanoic acid, Hexadecanoic acid, Octadecanoic acid
2	23.629	6.31	(E)-9-Octadecenoic acid ethyl ester, cis-13-Octadecenoic acid, Ethyl Oleate, methyl ester
3	23.755	2.91	Oleic Acid, cis-13-Octadecenoic acid, cis-Vaccenic acid
4	24.046	15.08	cis-Vaccenic acid, 9-Octadecenoic acid , cis-13-Octadecenoic acid

5	24.201	13.72	Oleic Acid, cis-Vaccenic acid
6	24.321	14.46	Oleic Acid, cis-Vaccenic acid
7	24.372	6.68	Oleic Acid , cis-Vaccenic
8	24.413	26.05	Oleic Acid, cis-Vaccenic acid, cis-13-Octadecenoic acid
9	31.142	3.52	Squalene

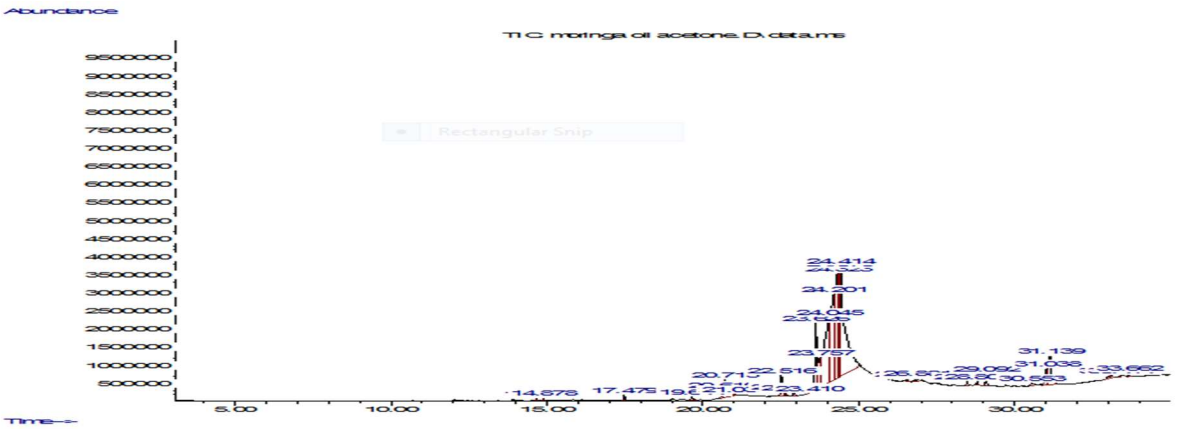
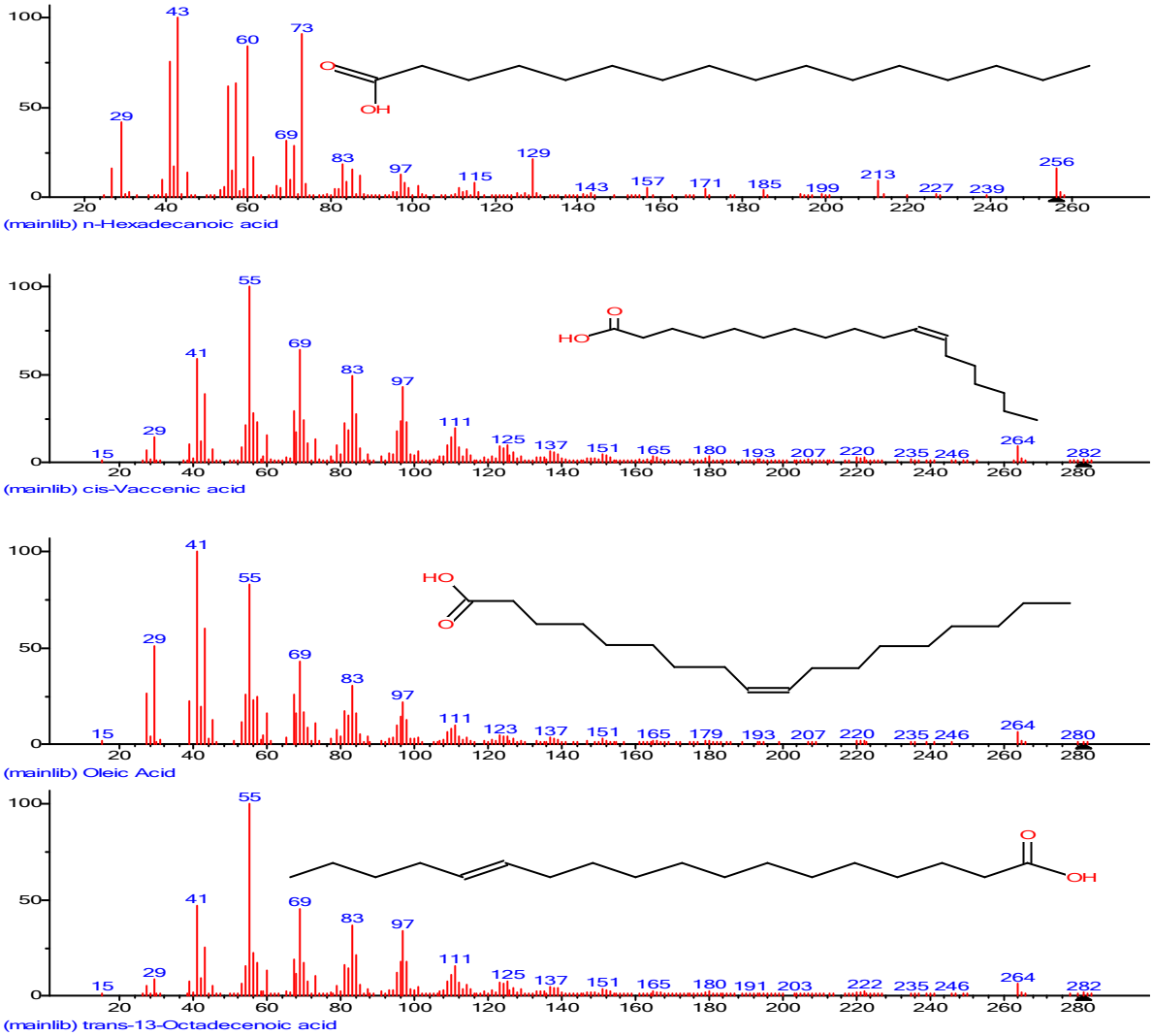


Fig-1: Spectrum for Acetone Extract



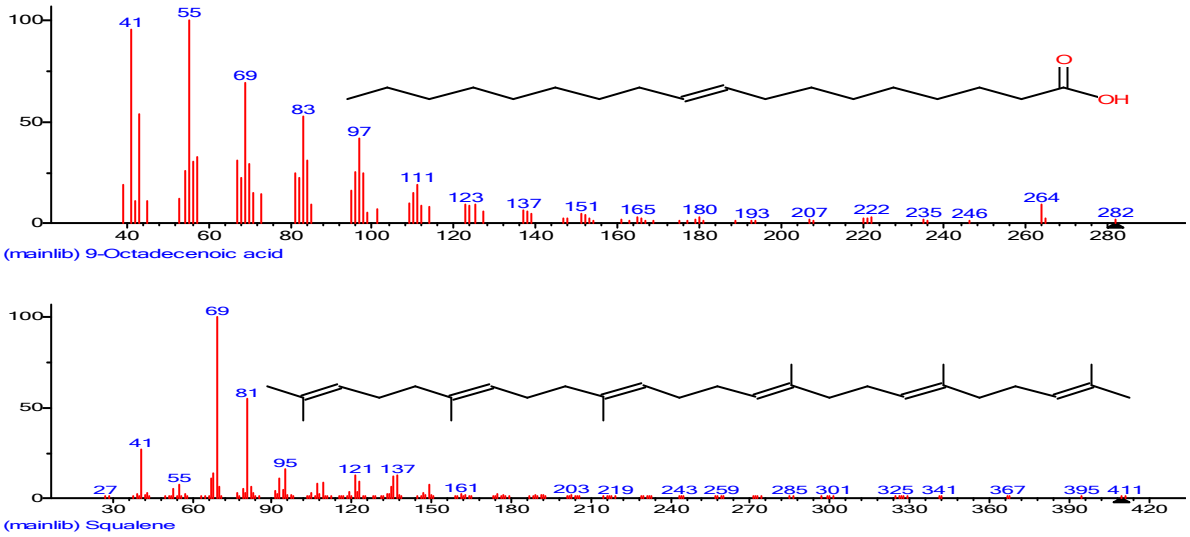


Fig-2: Mass Spectrum of Fragments of most abundant Chemical Constituents present in *M. oleifera* seed oil

Phytochemical Analysis

The phytochemical screening result in this research established the claims that *M. oleifera* has numerous health benefits, Table-1.[39] It was discovered that anthocyanin and betacyanin, quinones, Terpenoids, Tannins, Saponins and acids were all present in the samples analyzed. Other metabolites not present in this research have been identified by other researchers in different parts of *M. oleifera* plants (seeds, leaves, stem and flowers).[39-42] Several work have been carried out on the presence of phytochemical in different parts of plant.[41] Flavonoids are strong anti-inflammatory, antioxidants and antimicrobial properties *in vitro* against a broad group of microorganisms by inhibiting the bound membrane enzymes.[36,43,44] Aside possession of anti-oxidant and anti-inflammatory properties of the oil extract of *M. oleifera* seed, it was also reported that it possess substantial anti-mutagenic and anti-cancer activities.[45, 47-49] reported *M. oleifera* seed to be very good in controlling/reducing high blood pressure. Tannins are group of phenolic substances (polymeric)[50], inactivating and killing microorganisms.[36, 47] Anthraquinones and betacyanin belongs to the group of occurring (natural) phenolic compounds with laxative effects, offers anti-inflammatory, antiviral and anti-cancer benefits. Terpenoids is described as being active against bacteria such as *S. aureus*, fighting malaria and inflammation, capable of preventing cancer[51], having anti-carcinogenic effects.[52]

Antimicrobial Analysis

This was done *in vitro.* The presence of some phytochemicals in the seed of *M. oleifera* made it possible to have very good antimicrobial properties. Table-2 shows that oil extract was active against tested microorganisms, although very low. *B. subtilis, P. aeruginosa* and *S. aureus* had the same zone of inhibition (the highest); while *K. pneumoniae and E. coli* had the same zone of inhibition (lowest). The antibacterial activity of oil extracts was on the low side when compared to the zone of fungal isolates inhibition with 40 and 15mm for *C. albicans* and *R. stolonifera,* respectively.[53] Reported that the aqueous extracts and fresh leaf juice from *M. oleifera* seeds inhibit the growth of *S. aureus* and *P. aeruginosa.* The presence of flavonoids and tannins in the seed oil extract could be responsible for the antibacterial activity exhibited on some bacterial isolates since they have been reported to confer antibacterial activity.[36, 47, 54, 55]

MIC and MBC Analysis

Based on the MIC results MBC was performed against the entire selected microorganism using different concentrations of *M. oleifera* crude oil extract. The extract was discovered to have consequential antibacterial activity against microorganisms tested, the results of this research support the traditional claims of *M. oleifera* seed and it can be recommended for use as antimicrobial agent in new drugs for medical treatment of infectious disease caused by pathogens.

The result of the MIC of the extract showed that *B. subtilis, P. aeruginosa, S. aureus, E. coli, K. pneumoniae, C. albicans* and *R. stolonifera* were susceptible or sensitive at a concentration of 12.5 and 6.25mg/ml of the extract, bacterial growth was inhibited resulting in visually clear tubes after 24 hours of incubation. But *B. subtilis* was resistant (Table-3), the same trend was observed in MBC, Table-4.

Gas Chromatography Mass Spectrometry (GCMS)

About 33 chemical components were discovered and identified in the oil extract of *M. oleifera* seed. Only eight significant components with retention time varied from 20.710 – 31.142 were reported since they have significant quantity. Identified compounds were shown in table 5 with Oleic Acid, cis-13-Octadecenoic acid, cis-Vaccenic acid as the most abundant and occurrence. These are fatty acids found in oil and fats. These compounds are beneficial as a component in many foods industries. Oleic acid as one of its sodium salt is a significant component in producing soap as an emulsifying agent and can also be used as an emollient. Oleic, cis-13-Octadecenoic and cis-Vaccenic acid can be used as pharmaceutical solvents to decrease blood cholesterol levels and insulin, blood pressure and blood vessels and can also help to prevent heart disease and other related heart conditions.

CONCLUSION

From the result of the analysis, it can be concluded that oil extract of *M. oleifera* seed had broad-spectrum antibacterial activity and antifungal properties. The possible preventive and curative properties of the oil extract of *M. oleifera* seed were confirmed by the presence of phytochemicals. The sample analyzed using GCMS revealed the crude oil extract of *M. oleifera* to have various health benefits and can also be used in pharmaceutical industries. The results obtained in this research supported the claims that the seed of *M. oleifera* can be used in folkloric medicine as an antibiotic and in the treatment of other light aliments. It also represents an economic and safe alternative to treat infectious diseases in addition to its many other uses.

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