

SJMLS - 9(2) - 001

Proximate Analysis, Phytochemical Screening and Antimicrobial Evaluation of the Kernel and Oil Extract of *Moringa oleifera*

Akpo, Christiana Orevaoghene*¹, Ahomafor, Joy Emovigho², Nwankwo, Lawrence Uchenna*³, Ometie, Omaseye⁴
Department of Medical Laboratory Science, Faculty of Science, Delta State University, Abraka¹, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Delta State University, Abraka², Department of Pharmacognosy & Traditional Medicine, Faculty of Pharmacy, Delta State University, Abraka³, Delta State University Teaching Hospital, Oghara⁴

Author for Correspondence*: +234- 803-225-5534/ cakpo@delsu.edu.ng
<https://dx.doi.org/10.4314/sokjmls.v9i2.1>

Abstract

The aim of this study is to ascertain the phytochemical constituents, antimicrobial activity, and proximate analysis of the seed kernels of *Moringa oleifera*. The organisms tested for antimicrobial susceptibility were *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Shigella flexneri*, as well as the fungi *Aspergillus niger* and *Candida albicans*. *Moringa oleifera* seeds were gathered, dried in the air, shelled and ground into powder. The water content and ash value of the kernels were ascertained and were steeped in sterile water, then extracted. The seed oil was extracted using petroleum ether and a Soxhlet device. A standard analytical approach was utilized for phytochemical screening in order to assess secondary metabolites, and the agar well diffusion method was employed for antimicrobial evaluation. The mean ash value and moisture content of the seed were 50.3 ± 0.01 and $23.47 \pm 0.06\%$, respectively. The aqueous kernel extracts contained solely alkaloids, as identified by phytochemical screening of secondary metabolites. Conversely, the plant's oil extract did not contain any secondary metabolites. When testing the aqueous kernel extract against *P. aeruginosa*, the greatest zone of inhibition measured at 200 mg/ml was 19.7 mm. At 100, 50, 25, 12.5, and 6.25 mg/ml, the zones of inhibition measured were 16.3, 15.7, 11.3, 8.0 and 7.3 mm respectively. The outcome reveals that the kernel's aqueous extract had minimum inhibitory concentration (MIC) for *E. coli* and *P. aeruginosa* of 200 mg/ml and 6.25 mg/ml respectively. Based on the study's findings, it can be said that the whole kernel extract of *Moringa oleifera* has antibacterial capability and *P. aeruginosa* was most susceptible when compared with other species.

Keywords: Zone of inhibition, Agar well diffusion, minimum inhibitory concentration, phytochemical, antimicrobial

Introduction

Plants are a rich source of therapeutic compounds found in nature. It is well known that many contemporary medications have been generated from natural sources during the past millennium. The use of these agents in conventional medicine is the source of many of these compounds. Around 80% of the world's population receives their primary medical care mostly from traditional medicines, demonstrating the continued importance of natural products derived from plants in the provision of healthcare (Owolabi *et al.*, 2007). A number of the physical and chemical characteristics of natural products are investigated in an effort to assess the pharmacological or biological effects of crude medications. Similar to this, the variety of biological qualities displayed by natural goods can be used in pharmacy and medicine to increase the availability of therapeutic alternatives (Tasleem *et al.*, 2014).

Meanwhile, chemicobiology has been greatly impacted by the successful application of these concepts in the discovery of new medications, which has drawn attention to the use of natural products as biological function modifiers (Butler, 2008; Hong, 2011).

Globally, the food market segment with the quickest rate of growth is the functional food

market. A common example of a natural food source that can improve the functionality of other foods or operate as a functional food on its own is *Moringa oleifera*. It is asserted that the tree can boost food security, promote rural development, and enhance nutrition (Abou-Zaid and Nadir, 2014; Surbhi *et al.*, 2017). The popular household plant *Moringa oleifera* is well-known for its many culinary and medicinal applications. Domesticated throughout tropical and subtropical regions of the world, it originated on the Indian subcontinent (Farooq *et al.*, 2012). Common names for it include "drumstick tree", "benzoil tree", horse radish tree, miracle tree in English (USDA, 2014)

Other language names include: "Okweoyibo" in Igbo, "Zogeli" in Hausa and "Ewe igbale" in Yoruba speaking people of Nigeria (Anwar *et al.*, 2007). Conventional meals in tropical and subtropical regions include the leaves, flowers, fruits, and immature pods of the tree. The leaves are more incredibly rich in protein, iron and calcium, and vitamins A, B, and C than typical fruits like oranges, bananas, etc. (Kamal, 2008; Anhwange *et al.*, 2004). *Moringa oleifera* has amazing therapeutic potential and antibacterial properties. In autochthonous medicine, the root, leaf, bark, fruit, flowers, gum, seed, and seed oil have all been used to cure a variety of ailments (Farooq *et al.*, 2012). According to a report, *Lactobacillus sp.*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* cannot grow *in vitro* when exposed to an ethanol extract of *Moringa oleifera* seeds (Akpo *et al.*, 2021). According to Saadabi and Abu Zaid (2011), aqueous extracts of the plant have been reported to exhibit inhibitory efficacy against a variety of microorganisms, including *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Basidiobolus ranarums*. According to Siyanbola *et al.* (2015), *Moringa oleifera* seed oil's fatty acid profile makes it possible to mix it into beneficial organic coatings. The purpose of this study is to identify the phytochemical components and antibacterial activity of the kernel and oil extract of *Moringa oleifera* seeds against various microbial strains.

Materials and Methods

Plant Collection

The staff quarters in Campus II of Delta State University, Abraka, is where the *Moringa oleifera* seeds were found. They were identified, verified, and issued Voucher Number DELSUH086 at the Botany Department Herbarium, Delta State University, Abraka. The shell covering the kernels was removed after the seeds were taken out of the dried pods. Before grinding, the kernels were allowed to air dry for two weeks. They were then kept in a dark closet in an airtight container.

Preparation of Aqueous extract of M. oleifera Kernel

A slightly modified version of Obembe and Raji's (2018) approach was used. Six grams of powdered seeds were dissolved in thirty milliliters of sterile distilled water, and the mixture was thoroughly mixed for an hour and a half by stirring every thirty seconds with a spatula. The next step is to store it at 10 °C for three days. After being filtered via 2 µm pore filter paper, the mixture was consumed in 3 days.

Extraction of M. oleifera Kernel Oil

Using petroleum ether as the solvent, the oil was extracted using a Soxhlet extractor, and its characteristics were examined. A Soxhlet extractor with a 500 mL round-bottom flask and a condenser was fed with roughly 10g of ground seeds. Using 250 mL of petroleum ether, the extraction was carried out in a water bath for six hours. Once the extraction procedure was finished, a rotary evaporator was used to recover the oil. After one hour at 60°C in a hot air oven, the trace solvent was eliminated from the crucible. The extracted materials were refrigerated until they were needed for analysis.

Proximate Analysis

Moisture Content: The AOAC (2010) procedures were used to ascertain the samples' moisture content. A dried and weighed crucible was filled with around 2 g of the sample. At 70°C, the sample was left to dry until a consistent weight was achieved. After allowing the sample to cool inside the desiccators, it was weighed, and the moisture content was computed as a percentage.

Total Ash: A crucible that had been lit earlier was filled with precisely 1g of the pulverized, air-dried material. After evenly applying the substance, the heat was steadily increased to 400–500°C until the material turned white, signifying the absence of carbon. After cooling in a desiccator, the ash material was weighed. The total ash content was then calculated in mg per g of air-dried material.

Phytochemical Screening

Phytochemical Screening procedures were adopted from Oloye, (2005) to determine the secondary metabolites known as phytochemicals or bioactive constituents present in the plant sample.

Antibacterial activities

The bacterial isolates used for this study are *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella flexneri*. They were obtained from the Laboratory of the Department of Pharmaceutical Microbiology and Biotechnology and stored as stock cultures at 4°C in nutrient agar slants. Each isolate was sub-cultured in nutrient broth and after 24-hour incubation was adjusted to a concentration of 10^8 CFU/mL following bacterial plate counts.

The antimicrobial activities of the extracted oil, kernel and aqueous whole seed extract were determined respectively by the agar well diffusion method. Mueller Hinton agar was prepared following the manufacturer's instruction and poured in sterile Petri dishes to solidify. The plates were seeded with the respective standardized bacterial strains and punched with a sterile cork-borer followed by inoculation with different concentrations of the test solution and standard antibiotic, ciprofloxacin. Work was done in triplicates and plates were incubated at 37 °C for 24 h. The antibacterial activity was measured by measuring the zones of inhibition against the test organisms and the average noted.

Antifungal activity

Aspergillus niger and *Candida albicans*, the two fungal isolates employed in this investigation, were taken from stock cultures kept in the Pharmaceutical Microbiology Laboratory at a temperature of roughly -20°C. The isolates were incubated at room temperature for 72 hours after being subcultured in sterile Sabouraud Dextrose Agar. Filtration was used to create fungal spore suspensions, which were then diluted to 10^6 spores/ml to equal 0.5 McFarland's

standard. The agar well diffusion technique, as reported by Vollekova *et al.* (2001), was used to test the antifungals. The manufacturer's instructions were followed to create sterile Sabouraud dextrose agar, which was then put onto sterile petri dishes and allowed to set. Using a glass spreader, 100 microliters of the standardized fungal spores were equally distributed on the agar surface and left to dry. Wells were bored in the solidified agar using sterile corkborer. These wells were filled with 0.2 ml of the various concentrations of the extract and plates were incubated at room temperature for 120 hours. The sensitivity tests were done in triplicates. The zones of inhibition surrounding the agar wells were measured to determine antifungal activity. The average diameter of the zones of inhibition was recorded (CLSI, 2015).

Antibacterial Evaluation of Kernel Extracts of *M. oleifera* expressed as Minimum Inhibitory Concentration (MIC)

The examination of the oil and seed extracts' antibacterial properties was done, and the results were reported as Minimum Inhibitory Concentration (MIC). Using a two-fold serial dilution method, aqueous seed extracts were made into about six (6) concentrations (200 mg/mL, 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL). Six Petri dishes were utilized, separated into four sections for the organisms, and labeled with the *M. oleifera* seed extract preparation concentrations. The organisms were labeled on different divisions inside each of the six plates. The different concentrations were added to the correspondingly labeled plates. Nineteen milliliters of sterilized Mueller Hinton agar were added to the plate along with one milliliter of the plant extract. This was rocked gently to mix properly and allowed to solidify. After the organism was inoculated, the plate was incubated for twenty-four hours at 37°C. The Minimum Inhibitory Concentration (MIC) of the oil and seed extracts was determined to be the lowest concentration that prevented the isolate's growth. Using SDA as the medium, a similar procedure was done for fungi (CLSI, 2015).

RESULTS AND DISCUSSION

The proximate analysis, phytochemical screening and antimicrobial properties of seed, aqueous seed extract and oil extract of *Moringa oleifera* were investigated.

Table 1: Physicochemical Analysis of the Seed Kernel of *M. oleifera*

Parameters	Sample (Pulverized Seed)
Ash value (%)	50.3±0.01
Moisture Content (%)	23.47±0.06

Table 1 shows the result of the physicochemical analysis of the kernel of *Moringa oleifera* with a mean ash content of 50.3±0.01% and a percentage moisture content of 23.47±0.06%. The slightly high percentage moisture content of *Moringa oleifera* kernel is an indication of a relatively short shelf life of the crude drug since high moisture content provides a suitable condition for microbial growth on natural products. According to the Africa Pharmacopeia (1986), the moisture content of crude drugs must be equal to or less than 14%. The slightly high moisture content of the seeds of this plant has been shown to lower the oil yield of the plant (Adejumo *et al.*, 2013). The moisture content obtained in this study greatly contrasts with the 9.56% reported by Mgbemena and Obodo (2016). However, it is comparable to the 25.74% reported by Omofoyewa *et al.* (2017). An

increase in moisture content from 10.25 to 32.34% has reduced the crushing strength of the seeds of this plant (Ndukwe *et al.*, 2021).

Ash content determination is a form of physicochemical evaluation which ascertains the presence of minerals in a sample and the extent of foreign organic matter present in the crude drug. The high ash (50.3±0.01) content of the seeds of this plant is an indication that they are rich in minerals and may contain carbonates, phosphates, and silicates of sodium, magnesium, calcium and potassium. This result contrasts the 3.87±0.09%, 4.98% and 8.24% obtained respectively by Olagbemide *et al.* (2014); Compaoré *et al.* (2011) and Mgbemena and Obodo (2016). This variation may be due to the prolonged airing of the seed kernel which resulted in hygroscopy. The kernel may have absorbed some organic matter during the process.

Table 2: Phytochemical Evaluation of Seed Oil of *Moringa oleifera*

Phytochemical	Oil extract	Seed extract
Alkaloids	-	+
Flavonoids	-	-
Saponin	-	-
Steroids	-	-
Tannins	-	-
Cardiac glycosides	-	-
Terpenes	-	-
Reducing sugar	-	-
Anthraquinones	-	-

Key: Present (+) Absent (-)

The findings of the phytochemical screening of the *M. oleifera* kernel extract are displayed in Table 2. The outcome demonstrates that the oil extract included none of the secondary metabolites, but the seed aqueous extract only contained alkaloids and lacked the other metabolites. The results of the phytochemical screening of *M. oleifera's* seed extract and oil extract are displayed in Table 2. The seed extract solely reveals the presence of alkaloids, but the oil extract of the seed sample reveals

the absence of any secondary metabolites. This study's discovery of a single secondary metabolite is comparable to that of Bukar *et al.* (2010), who found that saponins were only present in the *M. oleifera* chloroform extract. This investigation differs from that of Ajayi and Fadeyi (2015), who found eight secondary metabolites in the *M. oleifera* seed extract. The existence of these secondary metabolites in the plant sample allowed for the assessment of the antimicrobial evaluation, which is why phytochemical constituents are significant in medicinal plant profiles.

Table 3: Sensitivity Test Evaluation of Kernel Oil Extract of *M. oleifera* against Test Organisms

Organisms	Concentration (mg/ml)						Control
	200	100	50	25	12.5	6.25	
<i>E. coli</i>	NI	NI	NI	NI	NI	NI	10.0
<i>P. aeruginosa</i>	NI	NI	NI	NI	NI	NI	32.3
<i>S. typhi</i>	NI	NI	NI	NI	NI	NI	11.3
<i>S. flexneri</i>	NI	NI	NI	NI	NI	NI	28.7
<i>C. albicans</i>	NI	NI	NI	NI	NI	NI	NI
<i>A. niger</i>	NI	NI	NI	NI	NI	NI	NI

Key:

NI = No inhibition

Control: Ciprofloxacin (2mg/ml)

Table 4: Sensitivity Test Evaluation of Kernel Devoid of Oil of *M. oleifera* against Test Organisms

Organisms	Concentration (mg/ml)						Control
	200	100	50	25	12.5	6.25	
<i>E. coli</i>	NI	NI	NI	NI	NI	NI	10.0
<i>P. aeruginosa</i>	NI	NI	NI	NI	NI	NI	32.3
<i>S. typhi</i>	NI	NI	NI	NI	NI	NI	11.3
<i>S. flexneri</i>	NI	NI	NI	NI	NI	NI	28.7
<i>C. albicans</i>	NI	NI	NI	NI	NI	NI	NI
<i>A. niger</i>	NI	NI	NI	NI	NI	NI	NI

Key:

NI = No inhibition

Control: Ciprofloxacin (2mg/ml)

Table 5: Mean Zone of Inhibition of Aqueous Kernel Extract of *M. oleifera* against Test Organisms

Organisms	Concentration (mg/ml)						Control
	Zone of Inhibition (mm)						
	200	100	50	25	12.5	6.25	
<i>E. coli</i>	11.0	10.3	10.3	9.0	6.7	5.7	10.0
<i>P. aeruginosa</i>	19.7	16.3	15.7	11.3	8.0	7.3	32.3
<i>S. typhi</i>	5.0	3.7	5.0	13.3	8.3	6.0	11.3
<i>S. flexneri</i>	NI	NI	NI	NI	NI	NI	28.7
<i>C. albicans</i>	NI	NI	NI	NI	NI	NI	NI
<i>A. niger</i>	NI	NI	NI	NI	NI	NI	NI

Key: NI = No inhibition

Control: Ciprofloxacin (2mg/ml)

Tables 3, 4, and 5 provide the results of the antimicrobial susceptibility test conducted on *M. oleifera* seed and oil extract, respectively. The outcome demonstrates that neither the oil extract nor the oil-free kernel showed any effect against the bacteria or fungi tested (Tables 3 and 4, respectively). At different doses, the aqueous whole kernel extract exhibited zones of inhibition against test microorganisms. At 200 mg/ml concentration, the maximum zone of inhibition against *P. aeruginosa* was 19.7mm. The following inhibitory zones were obtained at concentrations of 100, 50, 25, 12.5, and 6.25 mg/ml, respectively: 16.3, 15.7, 11.3, 8.0, and 7.3 mm. The ciprofloxacin zone of inhibition, which served as the standard was 32.3mm for *P. aeruginosa*. The lowest activity was seen against *S. typhi* with zones of inhibition of 5.0, 3.7, 5.0, 13.3, 8.3 and 6.0 mm at 200, 100, 50, 25, 12.5 and 6.25 mg/ml concentration respectively (Table 5). The antibacterial activity of *M. oleifera* kernel oil extract and kernel devoid of oil on test organisms demonstrates that neither material had any effect on the test organisms *in vitro*. This is explained by the lack of secondary metabolites, which are known to be in charge of the medicinal effects of crude pharmaceuticals. This investigation is consistent with that conducted by Patel and Mohan (2018), who found that the absence of secondary metabolites in the oil sample prevented the *M. oleifera* kernel oil extract from having any antibacterial action.

Salmonella typhi, *Escherichia coli*, and *Pseudomonas aeruginosa* are all susceptible to the effects of *M. oleifera* aqueous whole-seed extract (Table 5). The *P. aeruginosa* inhibitory zone was found to be the highest. The presence of alkaloids in the seed extract may be responsible for its action. This result is in contrast to the study by

Okafo *et al.* (2020), which reports that *Aspergillus flavus* and *Candida albicans* are sensitive to aqueous extracts of the plant's seeds, whereas *E. coli* and *P. aeruginosa* are resistant to them. Considering that the plants came from various sources, the age difference could be one of the causes of this. According to Farias *et al.* (2023), a plant's cytotoxicity reduced with age, and the age of the plant influences the essential oils' chemical makeup. Additionally, the results of this investigation do not agree with the work of Akpo *et al.* (2020), which demonstrated that aqueous stem bark extract of *M. oleifera* was not effective against *S. aureus*, *B. subtilis*, *Micrococcus*, and *E. coli*. This may be because the phytochemical composition of the two plant sections differs.

Singh and Bhat (2003) revealed that the antibacterial capabilities of plant extracts have been demonstrated to be enhanced by the presence of alkaloids, tannins, and flavonoids. This work bears similarities to those of Bukar *et al.* (2010), who found that *M. oleifera* exhibited antibacterial activity against *S. aureus*, *E. coli*, and *S. typhi*, with respective zones of inhibition measuring 10 mm, 7 mm, and 10 mm. In their work, Akhtar *et al.* (2007) documented the antibacterial properties of *M. oleifera* leaf and seed extract, with the extract demonstrating action against test organisms.

The seed extract's antifungal characteristic demonstrates that it was inert against *Aspergillus niger* and *Candida albicans*. This study supports the findings of Pate and Mohan (2018), who found that the seed extract had no effect on *Candida albicans* or *Aspergillus niger*.

Table 6: Antimicrobial Activity Expressed as Minimum Inhibitory Concentration (MIC) for Seed Extract of *M. oleifera*

Organisms	Concentration (mg/ml)						
	200	100	50	25	12.5	6.25	3.125
<i>E. coli</i>	-	+	+	+	+	+	+
<i>P. aeruginosa</i>	-	-	-	-	-	-	+
<i>S. typhi</i>	-	-	+	+	+	+	+
<i>S. flexneri</i>	+	+	+	+	+	+	+
<i>C. albicans</i>	+	+	+	+	+	+	+
<i>A. niger</i>	+	+	+	+	+	+	+

Key:

+ = Growth

- = No growth

The results of the antimicrobial activity of the seed extract, expressed as Minimum Inhibitory Concentration (MIC), are shown in Table 6. The minimum activity of the seed aqueous extract was 200 mg/ml for *E. coli*, 6.25 mg/ml for *P. aeruginosa*, and 100 mg/ml for *S. typhi*. The aqueous whole seed extract's MIC against *S. flexneri*, *C. albicans*, and *A. niger* indicates resistance to the test extract. The minimum inhibitory concentration (MIC) of the seed extract was observed at 200 mg/ml, 6.25 mg/ml, and 100 mg/ml against *E. coli*, *P. aeruginosa*, and *S. typhi*. This study demonstrates that the *M. oleifera* aqueous whole seed extract had better activity against *P. aeruginosa*, with the lowest MIC of 6.25 mg/ml compared to the other organisms. This runs counter to a report by Brooks *et al.* (2001), which stated that *P. aeruginosa* is recognized to be a resilient and challenging organism that causes issues for researchers.

Conclusion

Based on the study's findings, it can be said that the alkaloids found in the *Moringa oleifera* seed extracts employed in this experiment have antibacterial potential and are more effective against *P. aeruginosa* than other organisms. The plant seed extract's activity cannot be compared to that of prescription medications. The plant extract's minimal inhibitory concentration facilitates simple dose formulation and offers information about its potential chemotherapeutic indices in the event that it is employed as a substitute medication for *Salmonella typhi* and *Pseudomonas aeruginosa* infections. Nevertheless, there are instances where active ingredient extracts that worked well in *in vitro* tests could not work as well *in vivo*.

Recommendations

Authors hereby recommend further studies on the quantity of phytoconstituents present in the seed kernel and oil extract of *Moringa oleifera*. Moreso, more pathogenic microbes should be explored to ascertain the effect of the extracts under investigation against such life-threatening pathogens.

Acknowledgement

The authors acknowledge the effort of Mr. Evans Uvieghara and Mrs Helen Ayoola, the Laboratory Technologists in the Pharmaceutical

Chemistry Laboratory for assisting in the digestion of the samples and also for encouragement in carrying out this work.

Conflict of Interest

Authors hereby declare no conflict of interest.

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Citation: Akpo Christiana Orevaoghene, Ahomafor Joy Emovigho, Nwankwo Lawrence Uchenna, Ometie Omaseye. Proximate Analysis, Phytochemical Screening and Antimicrobial Evaluation of the Kernel and Oil Extract of *Moringa oleifera*. *Sokoto Journal of Medical Laboratory Science*; **9(2): 8–16**. <https://dx.doi.org/10.4314/sokjmls.v9i2.1>

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