

# Phytochemical and Antimicrobial Studies of Methanol, Ethyl acetate, and Aqueous Extracts of *Moringa oleifera* Seeds

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## ABSTRACT

The phytochemical properties and antimicrobial activities of extracts of *Moringa oleifera* whole seeds and dehusked seeds with methanol, ethyl acetate and water have been studied. The result of the phytochemical evaluation and thin layer Chromatogram showed that the extracts of the whole seeds compose of different secondary metabolites from the dehusked seeds. The antimicrobial study showed broad spectrum activities against both gram negative and gram positive organisms with zones of inhibition ranging from 9mm to 20mm against tested microorganisms. However the rate of inhibition increased in this order: methanolic extract > Ethyl acetate extract > aqueous extract.

**Keywords-** *Moringa oleifera*, Phytochemicals, Antimicrobial study, Extracting solvents.

## INTRODUCTION

*Moringa oleifera* (*M. Oleifera*) tree is a fast growing tree native to the Himalayas, however, is now grown in many warm regions of the world<sup>1,2</sup>. Various parts of *M. oleifera*, including young leaves, flowers, and green pods, are consumed for nutritional value. In addition, most parts of *M. Oleifera* have been used as traditional herbal remedies for the treatment of a variety of disorders such as skin diseases, respiratory sickness, ear and dental infections, hypertension, diabetes, anaemia, and

cancer<sup>1-3</sup>. The plant pod is reported to have to have antioxidant activity which is due to the presence of carotenoid compounds. It contains high amounts of bio-enhancer and can be used to reduce cholesterol and glucose in blood with safe intake level determined at < 1.000mg/Kg body weight<sup>4</sup>. The matured seed of *M. oleifera* is said to contain about 38-40% oil<sup>3,2,5</sup>. It is also used in the treatment of turbid water. Much has been reported on the physicochemical properties of the seed oil of *M. oleifera*<sup>3</sup>. The seeds are used as remedies for different disease and health conditions. The current

work is aimed at studying the phytochemical and antimicrobial effect of the extracts of *M. oleifera* seeds using different solvents in relation to its application in folkloric medicine.

## MATERIALS AND METHODS

The plant was authenticated by Professor O. Olorode Department of Biological Science, Botany Unit, University of Abuja, Nigeria. Matured seeds of the *Moringa oleifera* plant were harvested from the Chemistry Advanced Laboratory Moringa tree plantation and were identified by their physical properties as described by Price 2007<sup>14</sup>. They were removed from the pod and dried further. The seeds were prepared as both whole seeds and dehusked seed. The whole seeds were first milled into powder with the aid of an electrical grinder and stored in moisture – free container, then the dehusked seeds were also milled into powder and stored separately for comparison.

### Extraction of the plant sample

About 300 ml of distilled methanol, ethyl acetate and water each of the solvent was measured and added to the 100g of the blended mixture of husk and seeds first, also with seed only in a stoppered glass container. The mixture was left for three days for extracting and then finally filtered. The methanol, ethyl acetate and aqueous extracts for dehusked seeds and whole seed were concentrated on the water bath and stored for subsequent analysis.

### Phytochemical screening

For the purpose of this study, phytochemical screenings were carried out on the extracts to confirm the presence or absence of the following plant secondary metabolites: alkaloids, phenols, sterols, terpenes, tannins, flavonoids,

anthraquinones, cardiac glycosides, saponins, fats and oil<sup>6,7</sup>.

### Phenols

Equal volumes of each extract and ferric chloride solution (which is prepared by dissolving 135.2g of  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  in distilled water containing 20 ml of concentrated HCl dilute to 1 liter) are added together. A deep bluish green precipitate indicates the presence of phenol.

### Alkaloids

To each extract was added to 1% aqueous HCl over water bath and filtered. The filtrate was treated with (2g of Iodine in 6g of Potassium iodide in 100 ml of distilled water). Formation brown or reddish brown precipitate indicates presence of alkaloids.

### Steroids

Into each extract was added to 2ml acetic anhydride and 2ml  $\text{H}_2\text{SO}_4$ . Color change from violet to blue or green indicates the presence of steroids.

### Terpenes

To each extract was added to 0.5ml acetic anhydride and a few drops of concentrated  $\text{H}_2\text{SO}_4$ . A bluish green precipitate indicates the presence of terpenes.

### Cardiac glycosides

The extract was treated with 2ml glacial acetic acid with a drop of Ferric Chloride solution and underplayed with 1ml  $\text{H}_2\text{SO}_4$ . A browning at the interface indicates the presence of cardiac glycosides.

### Tannins

Each Extract was boiled in 20ml water and filtered. A few drops of 0.1% Ferric Chloride solution were added. Brownish green or blue-black color indicates the presence of Tannins.

### Flavonoids

5ml Ammonium solution was added to the aqueous filtrate of each extract and then a few drops of concentrated  $H_2SO_4$ . Yellow coloration indicates the presence of Flavonoids.

### Anthraquinones

10ml benzene was added to each extract and filtered. 0.5ml of 1% Ammonium solution was added and shaken. Pink, red, or violet color in the ammoniacal lower phase indicates the presence of Anthraquinones.

### Saponins

1g each extract was boiled with 5ml distilled water and filtered. 3ml distilled water was added to the filtrate and shaken vigorously for 5 minutes. Persistent frothing on warming indicates the presence of Saponins.

### Fats and oils

Small quantity of each extract was pressed between two filter papers. Oily stains indicate the presence of Fats and Oils.

### Triterpenoids

Crude extract was mixed with chloroform and few drops of conc.  $H_2SO_4$  was added, shaken and allowed to stand for some time. The formation of a yellow colored layer indicates the presence of triterpenoids.

### Glycosides

5ml  $H_2SO_4$  was added to each of the test extract in a boiling tube. The mixture was heated in boiling water for 15minutes. Fehling's solution A and B was added and the resulting mixture was heated to boiling. A brick red precipitates indicate the presence of glycosides.

### Carbohydrates

5ml of the equal mixture of both Fehling's solutions A and B was added to 2ml of test extract in a boiling tube, this was heated for 2 minutes. A brick red precipitates indicate a positive result.

### Phlobatannins

A few drops of 1% HCl were added to 1ml of test extract and was boiled. A reddish precipitates indicate the presence of phlobatannins.

### Resins

2ml of test extract plus an equal volume of acetic anhydride solution with drops of conc.  $H_2SO_4$  gives a colophony resin, a violet color indicates the presence of resins.

### Balsams

3 drops of alcoholic  $FeCl_3$  were added to 4ml of extract which was warmed. A dark green coloration indicates the presence of the balsams.

### Volatile oil

A small quantity of the test extract was shaken with dilute NaOH and 0.1M HCl. The formation of a white precipitate indicates a positive result.

### Antibacterial assays

The antibacterial assay was carried out using agar well diffusion tests. The antimicrobial activity of the plant extracts was tested on five bacterial clinical isolates namely; *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, the first four species represent gram negative bacteria while the last represent gram positive bacteria, these bacteria are the primary causative agents responsible for various human diseases conditions namely; urinary tract infections, arthritis and bone infections, diarrheal diseases, respiratory

infections and meningitis among others. The test organisms were gotten from patients attending Specialist hospital Gwagwalada, F.C.T. Abuja and were further isolated and characterized using various biochemical tests adopted from<sup>8</sup>.

#### Inoculum standardisation

The microorganisms were inoculated into Mueller Hinton broth and incubated at  $35 \pm 2^\circ\text{C}$  for 24 h. The turbidity of the suspensions scanned at 600 nm with an Ultra Violet Visible Spectrophotometer (Cecil, C-7500, Cambridge, England) the absorbance's were measured and diluted with normal saline to compare with 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately  $3.0 \times 10^8$  CFU/ml.<sup>9</sup>

#### Agar well diffusion assay

The agar well diffusion technique was the standard method used to determine the antibacterial activity of the bioactive compounds. The culture medium (Mueller Hinton agar) was prepared and treated according to the manufacture's instructions. The agar plates were incubated for 24 h at  $37^\circ\text{C}$  to confirm their sterility. Absence of growth after 24 h showed that the plates were sterile. The sterile Mueller Hinton agar plates were inoculated with the test culture by surface spreading using a sterile cotton board and each bacterium evenly spread on the entire surface of the plate to obtain uniformity of the inoculum. The culture plate, then had five wells of 8 mm diameter made into it using a sterile cork borer. Streptomycin was used as a positive control at a concentration of (25 $\mu\text{g}/\text{ml}$ ), while DMSO and distilled water (50:50v/v) was used as a negative control. Approximately, 0.2ml of the crude bioactive test compound of the various concentrations achieved using serial dilutions (50, 25, 12.5  $\mu\text{g}/\text{ml}$ ) were suspended in each wells and thereafter inoculated plates were incubated for 24 h at

$37^\circ\text{C}$ . The plates were examined for the presence of bacterial inhibition zones around each well. Antibacterial activities were determined by the presence of a zone of inhibition around the wells. The zones of inhibition were measured using a centimeter ruler and converted to the nearest millimeter (mm)<sup>16</sup>.

## RESULTS

The aqueous and methanol extracts of the samples gave brown sticky solids after drying while the ethyl acetate extract was purely oil. This indicates that the solvents extracted different components from the seeds as revealed in the phytochemical result in Table 1. The result indicates the presence of more secondary metabolites in the ethyl acetate and methanol fractions while the aqueous fraction of the whole seeds tested negative to most of the constituents analyzed. Tannins, phenols, carbohydrates, phlobatannins and resins tested negative in all the extracts.

The results of the antimicrobial assay reveal that the extracts possessed antibacterial properties in various degrees on test organisms. From Table 2 below, the dehusked seed methanolic extract shows great actions on all the gram negative bacteria tested. The highest zones of inhibitions were pronounced on *Proteus mirabilis* (20.0 $\pm$ 00mm) at 50 $\mu\text{g}/\text{ml}$  while the zone of inhibition of the remaining three gram negative organisms ranged between (18.0 $\pm$ 00 to 18.5 $\pm$ 00mm). The extract also shows activity on *Staph. Aureus* (14.5 $\pm$ 00mm) at 50 $\mu\text{g}/\text{ml}$  which qualified it as a broad spectrum extract. The methanol extract of whole seeds shows activities on all the bacterial tested with the highest zone of (17.5 $\pm$ 0.5mm) on *Proteus mirabilis* at concentration of 50 $\mu\text{g}/\text{ml}$ , the extract also possessed activity on *Staph aureus* at all the various working concentrations. The ethyl

acetate extracts of the dehusked and whole seeds on Table 4 and 5 showed that the activity of the extract range between (15.0±00 to 9.0±00mm) which indicated a weak activity when compared with that of methanol extracts on each test microorganisms. The aqueous extract of the dehusked seeds shows activities on only two test organisms *Proteus mirabilis* and *Klebsiella pneumonia* (9.0±00mm) at 50µg/ml each respectively. The result of the aqueous whole seed extract showed activities on *E. coli* and *Proteus mirabilis* in all the working concentration while no activities were recorded on *Staph. aureus*.

## DISCUSSION

The presence of some of these metabolites has been reported for the leaves. Compounds such as 4-(4'-*O*-acetyl- $\alpha$ -L-rhamnopyranosyloxy) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate and 4-( $\alpha$ -L-rhamnopyranosyloxy) benzyl glucosinolate, as well as carotenoids, niaziridin and niazirin, have been detected in *M. oleifera* pod<sup>4</sup>. An Ethanolic extract of the leaves showed the presence of thiocabamates such as niazinni A and B, and isothicyanates. The leaves also contain phenolic compounds, flavonoids, and glucosinolate<sup>10</sup>.

These chemical constituents are known for their many therapeutic values. Flavonoids are reported to have antifungal, anti-inflammatory, antibacterial properties<sup>11</sup> and anti-oxidant activities<sup>15</sup>. From the phytochemical screening, methanol and aqueous extracts of the dehusked seeds only and whole seed mixture showed the presence of steroidal compounds which are of great importance in pharmacy due to their relationship with sex hormones. It was reported by<sup>12</sup> that the leaves of *Moringa oleifera* are used as nutritional supplements for expectant mothers to ensure their hormonal balance, since steroidal structure

could serve as potent starting material in the synthesis of these hormones. The present result also suggests that the matured seeds may be used for the same purpose. The presences of terpene and triterpenoids were observed in the extracts. The role of terpenes and triterpenoid in biological activities has been reported in review and they have been reported to be significant in the treatment of cancer<sup>13</sup>.

From the result of the zone of inhibition in the microbial study, it was seen that both the extracts demonstrated antimicrobial activity. The methanolic extract demonstrated the higher activity with respect to the different concentrations followed by Ethyl acetate then Aqueous. It was reported that the pod extract of *Moringa oleifera* rich in niaziridin enhanced the bioactivity of several antibiotics (rifampicin, tetracycline and ampicillin) against bacteria and facilitated drug absorption through the gastrointestinal membrane<sup>4</sup>. A study reported that *Moringa* seeds were effective against skin infecting bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* in vitro evaluation<sup>14</sup>. Since activities were seen in both the methanolic, Ethyl acetate and water extract, it suggests that the crude extract can further be refined into pure form and use against pathogens that cause infections in local communities.

## CONCLUSION

The result of the analysis carried out supports the use of the seed of *Moringa oleifera* in folkloric medicine as an anti-biotic and in the treatment of other light ailments.

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**Table 1.** Results of phytochemical screening of methanol, ethyl acetate and aqueous extracts of dehusked and whole seeds

Test/samples	Ethyl. s	W. ethyl	Meth. s	Meth. w	Aq. s	Aq. w
Tannins	-	-	-	-	-	-
Steroids	-	-	+	+	+	+
Triterpenoids	+	+	-	-	-	-
Glycosides	+	-	-	-	-	-
Saponins	+	-	-	-	-	-
Phenols	-	-	-	-	-	-
Alkaloids	+	+	+	-	+	-
Terpenoids	-	+	+	+	+	+
Carbohydrates	-	-	-	-	-	-
Flavonoids	+	-	+	+	+	-
Cardiac glycosides	-	+	+	+	+	-
Phlobatannins	-	-	-	-	-	-
Resins	-	-	-	-	-	-
Balsams	-	-	-	+	-	-
Volatile Oil	+	+	-	-	-	-

**Note:**

Ethyl S = Ethyl Acetate extract (dehusked seeds), Ethyl W = Ethyl Acetate extract (Whole seed).

Meth. S = Methanol extract (dehusked seeds), Meth. W = Methanol extract (Whole seed).

Aq. S = Aqueous extract (dehusked seeds), Aq. W = Aqueous extract (Whole seed).

+ = Positive, - = Negative.

**Table 2.** Antimicrobial activity of the dehusked seeds methanolic extract

S/no.	Test organisms	Concentration in ( $\mu\text{g/ml}$ ) Zone of inhibitions are recorded in (mm).				
		50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	+ control (Strep).	-control (50:50). DMSO/DH <sub>2</sub> O
1	<i>Escherichia coli</i>	18.0 $\pm$ 00	17.5 $\pm$ 0.5	15.0 $\pm$ 00	23.0 $\pm$ 00	NA
2	<i>Pseudomonas aeruginosa</i>	18.0 $\pm$ 00	15.0 $\pm$ 0.5	11.0 $\pm$ 00	21.0 $\pm$ 00	-
3	<i>Proteus mirabilis</i>	20.0 $\pm$ 00	18.5 $\pm$ 0.5	13.5 $\pm$ 0.5	21.0 $\pm$ 00	-
4	<i>Klebsiella pneumonia</i>	18.5 $\pm$ 00	15.5 $\pm$ 0.5	11.0 $\pm$ 00	23.0 $\pm$ 00	-
5	<i>Staphylococcus aureus</i>	14.5 $\pm$ 00	9.5 $\pm$ 0.5	NA	20.5 $\pm$ 0.5	-

$\pm$  -The values are mean standard deviation of the duplicate.

$\mu\text{g}$ - Micrograms; Strep. – Streptomycin; DMSO- Dimethyl sulfoxide; DH<sub>2</sub>O- Distilled water;

NA- No activity.

**Table 3.** Antimicrobial activity of whole methanolic extract

S/no.	Test organisms	Concentration in ( $\mu\text{g/ml}$ ) Zone of inhibitions are recorded in (mm).				
		50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	+ Control (Strep).	-control (50:50). DMSO/DH <sub>2</sub> O
1	<i>Escherichia coli</i>	16.0 $\pm$ 00	11.0 $\pm$ 00	10.0 $\pm$ 00	22.5 $\pm$ 0.5	NA
2	<i>Pseudomonas aeruginosa</i>	13.5 $\pm$ 0.5	11.05 $\pm$ 0.5	9.5 $\pm$ 0.5	22.0 $\pm$ 00	NA
3	<i>Proteus mirabilis</i>	17.5 $\pm$ 0.5	11.0 $\pm$ 0	9.0 $\pm$ 00	21.0 $\pm$ 00	NA
4	<i>Klebsiella pneumonia</i>	16.0 $\pm$ 00	13.0 $\pm$ 00	11.0 $\pm$ 0.5	22.0 $\pm$ 00	NA
5	<i>Staphylococcus aureus</i>	16.0 $\pm$ 00	11.0 $\pm$ 00	9.0 $\pm$ 00	20.0 $\pm$ 00	NA

$\pm$  -The values are mean standard deviation of the duplicate.

$\mu\text{g}$ - Micrograms; Strep. – Streptomycin; DMSO- Dimethyl sulfoxide; DH<sub>2</sub>O- Distilled water; NA- No activity.

**Table 4.** Antimicrobial activity of dehusked seeds ethyl acetate extract

S/no.	Test organisms	Concentration in ( $\mu\text{g/ml}$ ) Zone of inhibitions are recorded in (mm).				
		50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	+ control (Strep).	-control (50:50). DMSO/DH <sub>2</sub> O
1	<i>Escherichia coli</i>	12.5 $\pm$ 0.5	11.0 $\pm$ 00	9.0 $\pm$ 00	21.0 $\pm$ 00	NA
2	<i>Pseudomonas aeruginosa</i>	11.0 $\pm$ 00	9.0 $\pm$ 00	NA	21.0 $\pm$ 00	NA
3	<i>Proteus mirabilis</i>	15.0 $\pm$ 00	13.0 $\pm$ 00	13.0 $\pm$ 00	21.0 $\pm$ 00	NA
4	<i>Klebsiella pneumonia</i>	14.0 $\pm$ 00	12.5 $\pm$ 0.5	11.0 $\pm$ 00	22.0 $\pm$ 00	NA
5	<i>Staphylococcus aureus</i>	11.0 $\pm$ 00	9.0 $\pm$ 00	NA	20.5 $\pm$ 0.5	NA

$\pm$  -The values are mean standard deviation of the duplicate.

$\mu\text{g}$ - Micrograms; Strep. – Streptomycin; DMSO- Dimethyl sulfoxide; DH<sub>2</sub>O- Distilled water; NA- No activity.

**Table 5.** Antimicrobial activity of whole seeds ethyl acetate extract

S/no.	Test organisms	Concentration in ( $\mu\text{g/ml}$ ) Zone of inhibitions are recorded in (mm).				
		50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	+ control (Strep).	-control (50:50). DMSO/DH <sub>2</sub> O
1	<i>Escherichia coli</i>	11.0 $\pm$ 00	9.0 $\pm$ 00	NA	22.0 $\pm$ 00	NA
2	<i>Pseudomonas aeruginosa</i>	NA	NA	NA	21.0 $\pm$ 00	NA
3	<i>Proteus mirabilis</i>	15.5 $\pm$ 0.5	13.0 $\pm$ 00	10.0 $\pm$ 00	21.0 $\pm$ 00	NA
4	<i>Klebsiella pneumonia</i>	12.0 $\pm$ 00	11.5 $\pm$ 0.5	10.0 $\pm$ 00	22.0 $\pm$ 00	NA
5	<i>Staphylococcus aureus</i>	NA	NA	NA	21.0 $\pm$ 00	NA

$\pm$  -The values are mean standard deviation of the duplicate.

$\mu\text{g}$ - Micrograms; Strep. – Streptomycin; DMSO- Dimethyl sulfoxide; DH<sub>2</sub>O- Distilled water; NA- No activity.

**Table 6.** Antimicrobial activity of dehusked seeds aqueous extract

S/no.	Test organisms	Concentration in ( $\mu\text{g/ml}$ ) Zone of inhibitions are recorded in (mm).				
		50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	+ control (Strep).	-control (50:50). DMSO/DH <sub>2</sub> O
1	<i>Escherichia coli</i>	NA	NA	NA	23.0 $\pm$ 00	NA
2	<i>Pseudomonas aeruginosa</i>	NA	NA	NA	22.0 $\pm$ 00	NA
3	<i>Proteus mirabilis</i>	9.0 $\pm$ 00	NA	NA	21.5 $\pm$ 0.5	NA
4	<i>Klebsiella pneumonia</i>	9.0 $\pm$ 00	NA	NA	22.5 $\pm$ 0.5	NA
5	<i>Staphylococcus aureus</i>	NA	NA	NA	20.0 $\pm$ 00	NA

$\pm$  -The values are mean standard deviation of the duplicate.

$\mu\text{g}$ - Micrograms; Strep. – Streptomycin; DMSO- Dimethyl sulfoxide; DH<sub>2</sub>O- Distilled water; NA- No activity.

**Table 7.** Antimicrobial activity of whole seeds aqueous extract

S/no.	Test organisms	Concentration in ( $\mu\text{g/ml}$ ) Zone of inhibitions are recorded in (mm).				
		50 $\mu\text{g/ml}$	25 $\mu\text{g/ml}$	12.5 $\mu\text{g/ml}$	+ control (Strep).	-control (50:50). DMSO/DH <sub>2</sub> O
1	<i>Escherichia coli</i>	11.0 $\pm$ 00	10.0 $\pm$ 00	9.0 $\pm$ 00	23.5 $\pm$ 0.5	NA
2	<i>Pseudomonas aeruginosa</i>	9.5 $\pm$ 0.5	NA	NA	22.0 $\pm$ 00	NA
3	<i>Proteus mirabilis</i>	12.0 $\pm$ 0.5	10.0 $\pm$ 00	9.0 $\pm$ 00	22.0 $\pm$ 00	NA
4	<i>Klebsiella pneumonia</i>	11.0 $\pm$ 0.5	9.0 $\pm$ 00	NA	23.0 $\pm$ 00	NA
5	<i>Staphylococcus aureus</i>	NA	NA	NA	20.0 $\pm$ 00	NA

$\pm$  -The values are mean standard deviation of the duplicate.

$\mu\text{g}$ -Micrograms; Strep.– Streptomycin; DMSO- Dimethyl sulfoxide; DH<sub>2</sub>O- Distilled water; NA- No activity.