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# Phytochemical Screening and Antimicrobial Activities of Ethanolic Extracts of *Moringa oleifera* Lam on Isolates of Some Pathogens

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#### Abstract

**Research Article** 

This work aimed to investigate the phytochemical compositions and in-vitro antimicrobial activities of ethanolic extracts of Moringa oleifera Lam against isolates of Staphylococcus aureus, Escherichia coli and Candida albicans. This involved phytochemical screening and antimicrobial testing of ethanol extracts of Moringa oleifera using basic pharmacognostic procedures and agar well diffusion assay on test pathogens respectively. Alkaloids, flavonoids, saponins and tannins were detected in all extracts with the exception of root which was devoid of saponins and the seeds which contained no tannins. The agar well diffusion assay showed that M. oleifera extracts showed antimicrobial activities against Escherichia coli, Staphylococcus aureus and Candida albicans. Minimum Inhibitory concentrations (MIC) values were (25 mg/ml and 50 mg/ml for the root), (100 mg/ml for the seed) and (50 mg/ ml and 100 mg/ml for the pod) against the three organisms. The leaf extracts was active against Escherichia coli and Staphylococcus aureus but not against Candida albicans. Standard Ciprofloxacin and Ketoconazole (controls) inhibited the test organisms by 100% at 50 mg/ml and 25 mg/ml concentrations respectively. The leaf extracts had the greatest antimicrobial activity against test bacteria (12 mm at 50 mg/ml) while bark extract had the least activity (8 mm at 50 mg/ml). However, only pod extract showed significant antifungal activity (10 mm at 50 mg/ml) while other extracts at the same concentration, showed no antifungal activity. Findings from this study revealed that ethanolic extracts of Moringa oleifera Lam exhibit significant antimicrobial activities on test pathogens and thus suggests need to refine and standardize these extracts as alternative source of antimicrobial medicines.

Keywords: *Moringa oleifera*; Antimicrobial; Phytochemistry; Pathogens

#### Introduction

As soon as man reached the stage of reasoning, he discovered plants that might be useful source of food, medicines, narcotics for religious rituals, or as poisons. Folk medicines, consisting largely of the use of plants, originated in this ideology and persisted to date. Among curative benefits derived from such practices were those gotten from the bark of the willow tree (febrifuge), now known to contain derivatives of salicylic acids; antihypertensive drug and tranquilizer, reserpine from the east Indian snake root, *Rauwolfia serpentine*, Chinese herb Manhung (*Ephedra vulgaris*) for treatment of asthma, active component of which is the alkaloid ephedrine; quinine from cinchona bark for treatment of malaria; and the heart drug digitalis from the purple foxglove [1].

Undoubtedly, most medicinal plants have beneficial values in substances present in their various tissues; these could elicit several physiological actions in the human body. Many of the plant species that provide medicinal herbs have been scientifically evaluated for their possible medicinal applications [2].

The "Moringa" tree is one of the world's most useful trees. In the tropics, it is used as forage for livestock, and in many countries, it is used as a micronutrient powder to treat various ailments [3]. The plant is highly valued since almost every part of the tree (leaves, roots, bark, fruit, flowers, immature pods and seeds) are used as food with high nutritional value [4,5]. In addition, the plant has been reported to possess antimicrobial properties and this explains the reason for its wide use in the treatment of human diseases [6].

Antimicrobials of plant origin have enormous therapeutic potential

in the treatment of infectious diseases and simultaneously mitigating most side effects that are often associated with synthetic antimicrobials [7]. Increase in incidence of multi-drug resistant bacteria has triggered immense interest in search of new drugs or alternatives from natural sources which include plants against this problematic group of pathogens such as the methicillin resistance *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa and Candida albicans* among others [8].

The specific components of Moringa preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include  $4-(4^{2}-O-acetyl-\alpha-Lrhamnopyranosyloxy)$ benzyl isothiocy-anate,  $4-(\alpha-L-rhamnopyranosyloxy)$ benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and  $4-(\alpha-L-rhamnopyranosyloxy)$  benzyl glucosinolate. It is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including  $\beta$ -carotene or provitamin A) [9]. Most of previous studies on *Moringa oleifera* showed different antimicrobial properties and phytochemical compositions.

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A disk-diffusion in-vitro antimicrobial activity of *Moringa oleifera* extracts against bacteria, yeast, dermatophytes and helminths was reported by Cáceres et al. The leaf juice and aqueous extracts from the seeds was shown to inhibit the growth of *P. aeruginosa* and *S. aureus*, however, no activity was demonstrated against other bacteria and *Candida albicans* [10]. However, Nwosu and Okafor reported antifungal activities of *Moringa oleifera* against seven pathogenic fungi using the broth dilution and agar plate methods [11]. More so, Spiliotis et al. reported antimicrobial activity from various varieties of *Moringa oleifera* seeds against some common clinical bacteria and *Aspergillus niger* [12].

This present study is essentially significant as the plant is readily available in our community and has been an edible delicacy by several people. Hence the rationale for this study is to establish the antimicrobial potentials of *Moringa oleifera* and support its use as alternative medicine for infections caused by *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* especially in resource limited settings.

# Materials and Methods

# Materials

These include test organisms (*Escherichia coli, Staphylococcus aureus* and *Candida albicans*), nutrient agar, 50 mg/ml ciprofloxacin and 20 mg/ml ketoconazole (positive controls), distilled water (negative control), syringes, test tube, beakers, conical flask, Bunsen burner, foil paper, cork borer (8 mm in diameter), refrigerator, meter rule, weighing balance, pestle and mortar, sieve, sample bottles and hand gloves.

# Reagents

Ethanol, sterile water, antiseptic liquid, 1% aqueous Hydrochloric acid, Meyer's reagent, Dragendorff's reagent, 1.8% aqueous sodium chloride, lead sub-acetate solution, 10% Ferric chloride.

# Collection, authentication and processing of plant materials

The fresh leaf, pod, root, bark and seed of *Moringa oleifera* Lam were collected from a garden at Gate 4, SSTH quarters university of Maiduguri. The plant materials were identified and authenticated by a botanical taxonomist Professor S. S. Sanusi of the Biological Science Department, University of Maiduguri. His analysis and description was in consonance with those found in various literatures [7,13]. The plant materials were dried under shade in the Pharmaceutics Laboratory, Faculty of Pharmacy, University of Maiduguri for about four weeks and then pounded into powdered form, using a mortar and pestle, and then sieved.

# Extraction

The powdered plant material (50 g each) of seed, root, bark, pod and leaf were soaked in 500 ml ethanol in separate 1 L capacity conical flasks stoppered and kept for 48 hours with intermittent shaking. The cold extracts thus obtained were filtered with Whatman's No 1 filter paper into evaporating dishes and allowed to dry at room temperature under normal atmospheric pressure. The extracts yielded were *Moringa oleifera* ethanol leaf extract 6.5 g (23.8%), *Moringa oleifera* seed ethanol extract 5.7 g (20.9%), *Moringa oleifera* pod ethanol extract 5.6 g (20.5%), *Moringa oleifera* root ethanol extract 4.8 g (17.6%) and *Moringa oleifera* bark ethanol extract 4.7 g (17.2%).

#### Phytochemical analysis

Phytochemical analysis for qualitative detection of alkaloids, flavonoids, tannins and saponins was carried out on the extracts as described by Evans [14] Sofowora [15] and Harbone [16].

**Test for alkaloids:** About 0.5 g of each extract was stirred with 2 ml of 1% aqueous hydrochloric acid on a steam bath and filtered. 1ml of the filtrate was treated with a few drops of Meyer's reagent and a second 1ml portion was treated similarly with Dragendorff's reagent. Precipitation with either of those reagents was taken as evidence for the presence of alkaloids [14,15].

#### Test for saponins:

• **Frothing test:** About 0.1 g of extract was shaken with 5 ml of distilled water in a test tube. Honey comb frothing that persisted for five minutes is taken as a preliminary evidence for the presence of saponins [16].

• **Heamolysis test:** About 2 g of the extract was added with 5 ml of distilled water and filtered. 2 ml of 1.8% aqueous sodium chloride was placed in two separate test tubes. To one of the tubes, 2 ml each of the filtrates was placed in one of the tubes containing 1.8% aqueous sodium chloride, while 2 ml of distilled water was placed in the other (containing 1.8% aqueous sodium chloride solution). 5 drops each of blood was added to the contents of the test tubes taking care not to shake the tubes. Hemolysis (settling down of the red blood cells) in the tubes containing the extracts and its absence in the control tubes was indicative of saponin [16].

#### Detection of flavonoids:

• Lead sub-acetate test: 0.5 g of extract was dissolved in 5 ml distilled water, heated for 5 minutes and filtered. The filtrate was allowed stand for 5 minutes to cool and about 2-3 drops of lead sub-acetate solution was added to the filtrate. A yellow precipitate indicated the presence of flavonoids [15].

• **Ferric chloride test:** About 0.1 g of extract was dissolved in 1 ml of ethanol; 1ml of 10% Ferric chloride was added. A brown solution with dirty green precipitate indicated the presence of flavonoids [16].

**Detection of tannins:** 0.5 g of plant extract was stirred with 1 milliliter of distilled water, filtered and few drops of ferric chloride were added to the filtrate. A blue-black, green or blue-green precipitate was taken as evidence for the presence of tannins [14-16].

# Sources of test organisms

Staphylococcus aureus (ATCC29213) and Escherichia coli (ATCC8739) were the test bacteria isolates, while Candida albicans (ATCC10231) was the test fungi isolate. These specimens were provided by MicroBiologics\* (Saint Claud, MN, USA) through Department of medical microbiology, University of Maiduguri Teaching Hospital. Cultural and morphological identification as well as biochemical confirmation of test specimen was done using protocols described by Cheesbrough [17]. Pure colonies of test organisms were maintained on nutrient agar media slopes for bacteria and Saboroud dextrose agar (SDA) slopes for Canida albicans.

#### Standardization and preservation of inoculum

The inoculum were prepared from the stock cultures, which were maintained on nutrient agar slant and SDA slopes at 4°C and sub cultured onto nutrient broth using a sterilized wire loop. The density of suspension inoculated onto the media for susceptibility test was

J App Pharm ISSN: 1920-4159 JAP, an open access journal determined by comparison with 0.5 McFarland standard of Barium sulphate solution. [17] Spore suspension for fungal bioassay was prepared according to the procedure of Murugan et al [18].

# Antimicrobial agar well preparation

A sterilized improvised cork borer of 8 mm in diameter was used to bore holes on the already prepared plates, the plates were bored to accommodate three (3) holes of 8 mm diameter each in which 0.5 ml of the three different concentrations [100 mg/ml, 50 mg/ml, and 25 mg/ml (for leaf, pod, and seed) and 50 mg/ml, 25 mg/ml and 12.5 mg/ml (for bark and root)] of the reconstituted plant extracts [of the 200 mg/ml stock solution (for leaf, pod, and seed) and 100 mg/ ml stock solution (for bark and root)] were administered, the plates were designed in such a way that each plate accommodated three holes each containing 0.5 ml of the smallest concentration, 25 mg/ml (for leaf, pod, and seed) and 12.5 mg/ml (for bark and root)] then the next plate accommodated three holes also, each containing 0.5 ml of the next geometric concentrations 50 mg/ml (for leaf, pod, and seed) and 25 mg/ml (for bark and root) up to the highest concentration (100 mg/ ml). Two plates containing three holes each was made for the positive controls (ciprofloxacin 50 mg/ml and 20 mg/ml ketoconazole) and negative control (Sterile water) 0.5 ml was administered.

#### Antimicrobial testing

Well agar diffusion technique described by Bauer et al [19] and demonstrated by Cakir et al [20] was employed for the antibacterial assay. Whereas, for antifungal susceptibility test, the extracts were incorporated into appropriate medium and subsequently fungal spore suspension inoculated. The preparation was incubated at appropriate temperatures. After incubation, zones of inhibition (diameter) formed on the medium were measured to determine antibacterial effectiveness of the different concentrations of the extracts, while sensitivity of the fungi to the test extract was recorded as described by Murugan et al [18].

# Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration for bacterial and fungal isolates were carried out using tube dilution technique as described by Akinyemi et al [21] and reported as the lowest concentration of the various extract that inhibit the growth of the test isolates. Stock solution (200 mg/ml) of the leaf, pod, and seed extracts were prepared by dissolving 2.5 g, 1.8 g and 1.9 g of leaf, pod, and seed extract in 12.5 ml, 9.0 ml, and 9.5 ml of sterile water respectively and stock solution (100 mg/ml) of the bark and root extracts were also prepared by dissolving 0.9 g and 1.0 g of bark and root extract in 9.0 ml and 10.0 ml of sterile water respectively. Serial dilution was carried out by adding 2.5 ml of nutrient broth at each dilution. Six concentrations were prepared from the stock solutions of leaf, pod and seed, such that each bottle contained 2.5 ml nutrient broth with extract concentrations that is equivalent to 100.000 mg/ml, 50.000 mg/ml, 25.000 mg/ml 12.500 mg/ml 6.250 mg/ ml and 3.125 mg/ml respectively. Same was equally done for the root and the bark, such that each bottle contained 2.5 ml nutrient broth and extracts concentrations of 50.0000 mg/ml, 25.0000 mg/ml, 12.5000 mg/ml, 6.2500 mg/ml, 3.1250 mg/ml and 1.5625 mg/ml, respectively. Small volume of the standard inoculums of each organism was then added to the bottle. The negative control contained the nutrient broth only, while the positive controls contained nutrient broth plus the test organisms. The bottles were labeled appropriately, and thoroughly mixed and incubated at 37°C for 24 hours for the bacteria and 48 hours for the fungal, after which they were examined for visible turbidity (growth). The minimum inhibitory concentration was reported as the lowest concentration that prevented visible growth [22].

# Determination of the minimum bactericidal/fungicidal concentration

This was determined by sub-culturing the last tube showing visible turbidity and all the tubes in which there is no visible turbidity unto fresh Mueller Hinton plate and incubated at 37°C for 24 hours for the bacteria and 48 hours for the fungal. All plates showing no growth on the Mueller Hinton agar indicated bactericidal effect of extract concentrations [22].

#### Statistical analysis

Data generated were systematically analyzed on Microsoft excel. Results were presented as either positive (presence) or negative (absent), frequencies and percentages in tabular forms. Pearson's correlation analysis was used to determine the inter-variable associations of various *Moringa oleifera* extracts on test pathogens. Thus, correlation graphs plotted.

# Results

Five (5) parts of different extracts of Moringa oleifera Lam viz; leaves (6.5 g), pods (11.2 g), barks (9.4 g), roots (9.6 g) and seeds (11.4 g) were dried, pounded and used for this study (Table 1). The results of the phytochemical analysis revealed varying constituents of these extracts. Alkaloids, flavonoids, saponins and tannins were detected in all extracts with the exception of root which is devoid of Saponins and the seed which contains no tannins (Table 2). The antimicrobial assay results revealed that M. oleifera pod; root and seed ethanol extracts exhibited antimicrobial activities against Escherichia coli, Staphylococcus aureus and Candida albicans (tables 3, 4 and 5). Minimum Inhibitory concentrations (MIC) values of Moringa Bark Extracts (MBE), Moringa Leaves Extracts (MLE), Moringa Pod Extracts (MPE), Moringa Root Extracts (MRE) and Moringa Stem Extracts (MSE) against Escherichia coli were 50.0 mg/dl, 12.5 mg/dl, 50.0 mg/dl, 25.0 mg/dl, 50.0 mg/dl (Table 3). Minimum Inhibitory concentrations (MIC) values of MBE, MLE, MPE, MRE and MSE against Candida albicans were 25.0 mg/dl, 25.0 mg/dl, 25.0 mg/dl, 12.5 mg/dl, 25.0 mg/dl (Table 5). Minimum Inhibitory concentrations (MIC) values of MBE, MLE, MPE, MRE and MSE against Staphylococcus aureus were 50.0 mg/dl, 25.0 mg/dl, 50.0 mg/dl, 25.0 mg/dl, 25.0 mg/dl (Table 3). The Moringa oleifera, leaf ethanol extract was active against Escherichia coli and Staphylococcus aureus but not against Candida albicans. Standard Ciprofloxacin and Ketoconazole (controls) inhibited the test organisms by 100% at 50 mg/ml and 25 mg/ml concentrations respectively (Tables 3, 4 and 5).

The MLE had the greatest antimicrobial activity against test bacteria (12 mm at 50 mg/ml) while MBE had the least activity (8 mm at 50 mg/ml). However, only MPE showed significant antifungal activity (10 mm at 50 mg/ml) while other extracts at the same concentration, showed no antifungal activity (Figures 1, 2, 3, 4 and 5).

S. No.	Initial amount of <i>M. oleifera</i> used (50 g)	Amount of extracts Recovered [g (%)]		
1	Leaf	6.5 (13.0)		
2	Seed	5.7 (11.4)		
3	Pod	5.6 (11.2)		
4	Root	4.8 (9.6)		
5	Bark	4.7 (9.4)		

 Table 1: Quantities of extracts recovered from 50 g M. oleifera Lam parts.

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Phytoconstituent	Method used	Bark	Leaf	Pod	Root	Seed
1.Tannins	Ferric chloride Test	+	+	+	+	-
2.Flavonoids	Lead sub-acetate Test Ferric Chloride Test	++++	++++	+ +	+ +	+ +
3. Alkaloids	Wagner's Test Mayer's Test Dragendorff's Test	++++++	+ + +	+ + +	+ + +	+ + +
4. Saponins	Frothing Test Heamolysis test	++++	++++	+ +	-	+ +

Key: (+) = present, (-) = absent

Table 2: Preliminary phytochemical constituent of ethanolic extracts of Moringa oleifera Lam bark, leaf, pod, root and seed.

Conc. of the Extracts (mg/ml)	Average Zone of Inhibition (mm)								
	MBE	MLE	MPE	MRE	MSE	+ve Control	-ve Control		
100	-	16.3	14.3	-	11.0	-	-		
50	8.0	12.0	10.3	11.3	9.0	38.0	8.0		
25	8.0	8.0	8.0	11.0	8.0	-	-		
12.5	8.0	-	-	8.0	-	-	-		
MIC (mg/ml)	50.0	25.0	50.0	25.0	25.0				
MBC (mg/ml)	>50	>100	>100	>50	>100				

Positive control=Ciprofloxacin (50 mg/ml), Negative control=Distilled H<sub>2</sub>O, p-value <0.05 is considered significant, the highest concentrations were compared with the standards using Student's t-test, Diameter of the Cork Borer=8 mm.

Table 3: Antimicrobial analysis of the ethanol extract of Moringa oleifera Lam on Staphylococcus aureus.

Conc. of the Extracts (mg/ml)	Zone of Inhibition (mm)							
	MBE	MLE	MPE	MRE	MSE	+ve Control	-ve Control	
100	-	14.0	13.7	-	10.0	-	-	
50	8.0	12.0	10.7	10.8	9.0	38.0	8.0	
25	8.0	8.0	8.0	10.6	8.0	-	-	
12.5	8.0	-	-	8.0	-	-	-	
MIC (mg/ml)	50.0	12.5	50.0	25.0	50.0			
MBC (mg/ml)	>50	>100	>100	>50	>100			

Positive control=Ciprofloxacin (50 mg/ml), Negative control=Distilled H<sub>2</sub>O, p-value <0.05 is considered significant, the highest concentrations were compared with the standards using Student's t-test, Diameter of the Cork Borer=8 mm.

Table 4: Antimicrobial analysis of the ethanol extracts of Moringa oleifera Lam on Escherichia coli.

Conc. of the Extracts (mg/ml)	Zone of Inhibition (mm)								
	MBE	MLE	MPE	MRE	MSE	+ve Control	-ve Control		
100	8.0	8.0	11.7*	9.0	9.0	-	-		
50	8.0	8.0	10.0	8.7	8.0	-	-		
25	8.0	8.0	8.0	8.0	8.0	13.7	8.0		
12.5	8.0	-	-	8.0	-	-	-		
MIC (mg/ml)	25.0	25.0	25.0	12.5	25.0				
MBC (mg/ml)	>50	>100	>100	>50	>100				

Positive control=Ketoconazole (25 mg/ml), Negative control=Distilled  $H_2O$ , p-value<0.05 is considered significant, the highest concentrations were compared with the standards using Student's t-test, p-value is >0.05, so it not statistically significant. Diameter of the Cork Borer=8 mm

Table 5: Antimicrobial analysis of the ethanol extract of Moringa oleifera Lam parts on Candida albicans.

# Discussion

The result of phytochemical screening in this study revealed the presence of alkaloids, flavonoids, saponins and tannins in all extracts, with exceptions of the root (absence of saponins) and the seed (which was devoid of tannins).

The phytochemical constituents observed on the leaf extract in this study have been documented to be the major bioactive plant ingredients as well as exhibiting physiological activity [23]. This was also in agreement with work of Aliyu et al [24] and Dahiru et al [25] however Bukar et al [26] reported contrary result, showing lack of alkaloids and tannins in the ethanol extract of the leaf. This could be due to the development stage of the specimen used and their age.

Alkaloids were present and lack of tannin in the seed agree which the work of Bukar et al [26] but this was not determined by Napolean et al [27] however goes contrary with the findings of Kawo et al [28] which reveal presence of tannins and saponins in the ethanol seed extract. The same reasons, the difference could be attributed to variation in the environment where the plant was collected, the season and the physiological stage of the plant when leaves were harvested.

Kasolo et al [29] reported the presence of alkaloids in the ethanol

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Figure 2: Graph of diameter of zone of inhibition of micro-organisms against concentration of ethanol extract of *Moringa oleifera* leaf.









extract of root which agrees with our findings. Saponins could not be detected in the root contrary to the report of Kasolo et al [29]. However, in their finding they reported absence of Gallic tannins (a type of tannin), while tannin was present in the root in our finding but it was not characterized. This could be due to chemical races, or geographical effect.

From the results of antibacterial activity of *Moringa oleifera* extracts, it can be deduced that *Moringa oleifera* leaf ethanol extract had the broadest spectrum of activity on the test bacteria but no activity on the fungus. The results show that it had activity against the two bacterial isolates. *Staphylococcus aureus* (16.3 mm) and *Escherichia coli* (14.0 mm) were sensitive at concentration of 100 mg/ ml, but were not sensitive at the concentrations 25 mg/ml used. Similar studies conducted by Bukar et al [26] and Napolean et al [27] reported that ethanol leaf extracts were sensitive to *S. aureus* and *E. coli* at concentration of 200 mg/ml. However, a contrary finding was reported by Arzai, [30] where no antimicrobial activity was obtained at higher concentration of 250 mg/ml. Findings by Maroyi [31] also found that leaf extract has antibacterial activity against *Staphylococcus aureus*.

Findings from our study revealed that *Moringa oleifera* seed ethanol extract was active against bacterial isolates and the fungal isolate with *Staphylococcus aureus* (11 mm) and *Escherichia coli* (10 mm) and *Candida albicans* (9 mm) at concentration of 100 mg/ml. The

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antibacterial activity at theses concentrations of *Moringa oleifera* seed have been reported by some authors [32-34].

The antibacterial properties of the leaf and seed of *Moringa oleifera* as shown in the present study was in conformity with earlier findings by Akhtar et al [34] and Foidl et al [35] who reported antibacterial properties of seed and leaves extracts of *Moringa oleifera*.

The antimicrobial activity of *Moringa oleifera* seed is due to the presence of a significant phytochemical of a short polypeptide called 4 ( $\dot{\alpha}$  – L – rhamnosyloxy) benzyl-isothiocyanate [36,37]. This peptide act directly on bacteria and result in growth inhibition by disrupting cell membrane synthesis and / or synthesis of essential enzymes [38,39].

The antifungal activities of *Moringa oleifera* seed and leaf extracts against *Candida albicans* in our finding was contrary to finding reported by Nwosu et al and Raheela et al [11,40] because they demonstrated that leave and seed extract to be active on *Rhizopus spp.* and Gopalakrishna et al [41] reported pterygospermin as having high activity against some moulds and fungi. More so, Donli et al. and Chuang et al. reported antifungal activities *in vitro* against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum* and *Microsporum canis* from ethanol extracts and essential oil of *Moringa oleifera* [5,42].

The pods extract shows activity on the fungal isolates (11.7 mm) at concentration of 100 mg/ml, while the root extract was active (8.7 mm) at a lower concentration of 50 mg/ml. The zone of inhibition (13.7 mm) of the positive control ketoconazole 20 mg/ml does not differ much with that of the extracts. This suggests that the pod extract will have greater activity if purified.

Flavonoids and tannins have been reported to possess antimicrobial activity, the antimicrobial activity of flavonoids is due to their ability to complex with extracellular and soluble protein and to complex with bacterial cell wall while that of tannins may be related to their ability to inactivate microbial adhesions, enzymes and cell envelope proteins. The presence of alkaloids, tannins and flavonoids in plants extracts has also been shown to enhance antimicrobial properties as reported by Singh and Bhat [43] and Tscheche [44].

The leaves of *M. oleifera* have also been known to contain a number of phytochemicals such as flavonods, saponins and tannins that exhibit antimicrobial activities [45,46]. This suggests that the antimicrobial activities demostrated in this study could be attributed to these compounds. The mechanisms of actions of these compounds have been proven to be through cell membranes perturbations [47]. This alongside with the action of  $\beta$ -lactams on the transpeptidation of the cell wall could lead to an enhanced antimicrobial effect of the combinations [47].

## Conclusions

The ethanol extracts of all the parts of *Moringa oleifera* used in this experiment showed variable and significant antimicrobial activity against test pathogens, this thus supports the fact that *Moringa oleifera* contains active phytochemicals that exhibit antimicrobial potentials.

The information provided by this study on various MICs of these extracts will make it easy for dosage formulation and insight into their chemotherapeutic indices if they were to be used as alternative medicines for infections caused by *Staphylococcus aureus, Escherichia coli* and *Candida albicans.* However in some cases, extracts of active constituents that were effective during *in-vitro* experiments may not necessarily have equal potency when applied *in-vivo*.

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