



Original article

Antibacterial activity of *Moringa oleifera* methanolic leaves extracts against some Gram-positive and Gram-negative bacterial isolates

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ABSTRACT

Background: *Moringa oleifera* Lam. (Moringaceae) found to be very useful tree in tropical countries. In folklore and Ayurvedic all parts of the tree are used in different healing procedures for different diseases. The plant leaves are very good nutrient supplement for malnutrition and also used as an antibiotic. **Methods:** Phytochemical analysis of the leaf in solvents of varying polarity; viz., aqueous and methanol were also carried out. **Results:** The phytochemical screening indicated the presence of phenolics, flavonoids, tannins, glycosides etc. The antibacterial activity of *Moringa oleifera* leaf extracts against four microorganisms, viz. *Escherichia coli*, *Shigella*, *Staphylococcus aureus* and *Streptococcus*; the methanolic extract was active against *E.coli*, *Shigella*, *Staphylococcus aureus* whereas the aqueous extract exhibited an inhibitory effect on *E. coli* and *Shigella* only at different zone of inhibition levels of extracts. Well diffusion method was used to assess the antibacterial effect of the extracts on both Gram positive and Gram negative micro-organisms. Moreover, statically, the results were not significant at both 1% and 5% level of significances. It became obvious that, the higher reducing power of the aqueous extract could be due to the better solubility of the antioxidant components in water whereas the predominant antibacterial activity in organic solvent extracts as compared to aqueous extracts, indicated that the active components responsible for the bactericidal activity are more soluble in organic solvents. **Conclusion:** This study provided an evidence to support traditional medicinal uses of the plant.

Introduction

The *Moringa* plant (*Moringa oleifera*) has been known to many, that it cures so many bacterial infections due to the multiple researches surfaced nowadays [1]. *Moringa oleifera* is an edible as well as fastest growing tropical plant. It is utilised in many parts of the world due to its ability in addressing so many abnormalities. It has been mentioned in ancient Egyptian, Romans and Greeks. Many parts of African, Asian, Latin America and Caribbean countries utilized

it for problems solving [2]. *Moringa oleifera* (*Moringaceae*) has a clear attribute to both medicinal and nutritional quality. The plant has enough natural compounds which made it rich in minerals, vitamins, carbohydrates etc. *Moringa oleifera* is highly blessed for having a power of purifying water as well as extracting valuable natural products for the use as medicines [3]. Different parts of this plant such as the bark, leaves, immature pods, roots, fruit, flowers and seeds serve as cardiac and circulatory stimulants,

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possess antitumor, antipyretic, antiepileptic, cholesterol lowering, antihypertensive, anti-inflammatory, antispasmodic, antiulcer, diuretic, antifungal, antibacterial hepatoprotective, antioxidant, antidiabetic activities. Traditionally, it served as a treatment for different ailments in medical system as stated by **Guevara et al.** [3].

It has been proved that it has a numerous activity since before now; it inhibits the growth of fungi [4], as well as proved to have antibacterial effect against *Staphylococcus aureus* [5]. The fresh leaf juice was helpful to inhibit the growth of microorganisms that are pathogenic to man such as; *Staphylococcus aureus* and *Pseudomonas aeruginosa* [6]. The aqueous extracts of *Moringa oleifera* was reported to have potentiality against many pathogenic bacteria, such as; *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* [7].

Moringa oleifera also has a rich mine of antioxidant [8]. The antioxidant properties in the aqueous extracts of leaf, fruit and seeds of *Moringa oleifera* was already presented by **Singh et al.** [9]. Antioxidant property of freeze dried *Moringa* leaves extracted from different procedures, gave an idea that Indian origin's methanol and ethanol extracts of *Moringa oleifera* have the highest antioxidant activity of 65.1 and 66.8%, respectively [10,11]. In addition to this information, **Lalas and Tsaknis** [11], concluded that the major bioactive compounds of phenolics, like quercetin and kaempferol are attributes for antioxidant activity. Quercetin and kaempferol are also reported to have good antioxidant activity on hepatocyte growth factor induced Met phosphorylation with IC50 value for 12 and ~6 $\mu\text{m/L}$, respectively [12]. The objectives of this research include (i) To determine the activity of *Moringa oleifera* leaves extract against the bacterial isolates. (ii) To isolate and characterized both the Gram-negative and Gram-positive bacterial isolates from samples collected and (iii) To determine the minimum inhibitory concentration (MIC) of ethanolic extract of *Moringa* leaves extract against some Gram positive and Gram-negative bacteria.

Materials and Methods

Collection and identification of plant material

The leaves of *Moringa oleifera* tree were collected from study area. The leaves were identified taxonomically deposited at the botanical garden Department of Biology, Yobe State University. It was ensured that the plant was healthy and uninfected. The leaves were washed under running tap water to

eliminate dust and other foreign particles so as to be clean respectively.

Preparation of plant material

The leaves were air dried and grounded with the aid of clean mortar and pestle into a coarse powder, sieved with 1mm² and stored in a plastic container as described by **Abdallah et al.** [13]. About 60g of the powdered form of the leaves of *Moringa oleifera* was suspended in 500ml of ethanol and distilled water in separate, whereby, the suspensions were kept at room temperature and about 24 hours with constant shaking. The suspensions were then filtered, and solvent removed by evaporation to dryness at room temperature [13].

Phytochemical screening

The collected plant extracts were subjected to qualitative phytochemical analysis for identification of various classes of active chemical constituents, carried out using standard methods.

Test for alkaloids (Mayer's test)

To 1 ml of leaf extract, 6 drops of Mayer's reagent was added. The formation of yellowish precipitate indicated the presence of alkaloids [14].

Test for saponins (Foam test).

One ml of leaf extracts was mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins [14].

Test for tannins (Braymer's test)

One ml of the leaf extract was mixed with 2ml of water. To these, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [14].

Test for steroids (Salkowski test)

To 2ml of the leaf extract, 2ml of chloroform was added followed by concentrated sulphuric acid. Formation of reddish-brown ring at the junction showed the presence of steroids [15].

Test for terpenoids

To 2ml of the leaf extract 2ml acetic acid was added. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids [14].

Test for phenols

One ml of the leaf extracts was treated with 3% ferric chloride. The appearance of deep blue color shows the presence of phenol [16,17].

Test for flavonoids

One ml of the leaf extracts was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [16, 17].

Test for quinones

One ml of the leaf extracts was treated with 5ml of HCL. Formation of yellow color precipitate indicates the presence of quinones [16,17].

Test for phytosterols (Salkowsis test)

Extracts were treated with chloroform and filtered, then treated with few drops of concentrated sulphuric acid, Shaken and allowed to stand. Golden colour appearance indicates the presence of phytosterols [16, 17].

Test organisms

The clinical isolates were obtained from stool samples collected from Yobe State Specialist Hospital Damaturu. The organisms include; *E. coli*, *Shigella* spp, *Streptococcus* spp, *staphylococcus* spp etc. The isolates were identified using the schemes of **Cheesbrough** [18]. Then, sub-cultured into MacConkey agar, Eosin methylene blue and Salmonella – Shigella agar for further confirmation [18].

Culturing and isolation of the test organisms

A sterile wire loop has been used to inoculate stool samples on nutrient agar. The culture was then incubated at 37 °C for 24 hours. The colonies showed the presence of both *Shigella*, *Salmonella*, *E. Coli* *Streptococcus* and *Staphylococcus*. *Shigella*, *Salmonella* and *E. coli* are all Gram-negative bacteria while *Streptococcus* and *Staphylococcus* are Gram positive bacteria [18]. However, during inoculation, the plates of the media were dried to allow easier growth and identification of the colonies. The wire loop was also flamed and sterilized. The plates were placed inverted overnight, to prevent falling of condensed water vapour on plate surface [19]

Gram staining technique

Thin smears of about 20mm in diameter were made on clean grease free slide then fixed over a burning flame. A crystal violet solution was applied to cover the smear for 30 seconds after that it was washed with distilled water. Secondly lugol's iodine was also applied to the surface for another 30 seconds, acetone was also used to decolorize the stain, lastly the neutral red solution was covered on the surface for a minute, which was washed up and it was also allowed to dry at room temperature. Stains were observed under microscope with oil immersion. Red stain indicated Gram-negative bacteria [19].

Biochemical test**Indole Test**

Tryptophan broth was inoculated with an isolate of the test organism and incubated at 37°C for 24 hours. About 0.5ml of Kovack's reagent was added to the broth culture [20]

Methyl red Test

MR-VP broth was inoculated with an isolate of the test organism using sterile inoculating loop and incubated at 37°C for 24 hours. About 5 drops of methyl-red reagent was added to the broth culture [20].

Voges Proskauer

MR-VP broth was inoculated with an isolate of the test organisms using sterile inoculating loop and incubated at 37°C for 24 hours. Six millilitre (6ml) of 5% alpha naphthol was added followed by 0.2ml of KOH. The tube was shaken gently and remained undisturbed for 5 minutes [20,21]

Citrate utilization test

Simmons's citrate agar was streaked back and forth with inoculums of the test organism and incubated aerobically at 37°C for 24 hours [20].

Urease test

Urease agar medium was prepared and sterilized using autoclave. The medium was allowed to solidify in the slanting position to form a slope. The slants were inoculated with test organism. The tubes were incubated at 37°C for 24 to 48 hrs. The slants were observed for colour [20].

Sensitivity testing

Mueller Hinton agar (Fluka) was prepared, based on the manufacturer's guide and suspended into a clean conical flask containing 1litre of sterilized distilled water and allowed to sock and dissolved for some minutes, boiled for some minutes then autoclaved at 121°C for 15 minutes. Furthermore, each organism (culture) was inoculated on plates using swab stick. A 6mm cork borer was used to bore holes on the medium. Six holes were made on each petri plates, adequately spaced out. About 0.2 ml of the different concentrations (25, 50,100,150 and 200mg/ml) were introduced into the well. The petri plates were incubated at 37°C for 24hrs, after which the zones of inhibition were measured using a meter rule [21].

Determination of minimum inhibitory concentration

Minimum inhibitory concentration of the extract of the bacterial test organisms was determined by broth dilution method. Test tubes were labelled and 5ml of nutrient broth was introduced into each test tube,

0.5ml of bacterial suspension was inoculated. Followed by the addition of different concentrations (25mg/ml, 50mg/ml, 100mg/ml, 150mg/ml, and 200 mg/ml). The mixtures in all test tubes were mixed properly before incubation at 37°C for 24hrs. Observation for turbidity was carried out. The turbidity shows bacterial growth. The MIC was

determined by the lowest concentration of the extract that prevented visible growth [22].

Statistical analysis

The package used for data analysis was Statistics 8.0 so as to know the level of significant. Source (SAS)

Results

Table 1. The physical characteristics of both methanolic and aqueous extract of *Moringa oleifera*.

Extract	Weight Cone (g)	% Yield	Characteristics texture	Appearance
Methanolic extract of <i>Moringa oleifera</i>	60	113.0	Oily	Green colour
Aqueous extracts of <i>Moringa oleifera</i>	60	195.5	Gummy	Dark green colour

Table 2. Qualitative analysis of phytochemical screening of *Moringa oleifera*.

SN	Phytochemical ingredient	Leaves status
1	Alkaloids	+
2	Saponins	-
3	Phytosterols	+
4	Phenols	+
5	Tannins	+
6	Steroids	-
7	Terpenoids	-
8	Quinones	-

+ = Present, - = Absent

Table 3. Morphological and biochemical test for identification of the isolates.

Biochemical test	<i>E. Coli</i>	<i>Shigella</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>
Colony morphology on nutrient agar	Green pinpoint colonies	Cream colonies	Cream coloured	Cream coloured
Colony morphology on selective medium	EMB agar, shining greeny metallic	XLD agar colonies	Catalyst agar hydrogen peroxide	Catalyst agar hydrogen peroxide
Gram nature	Gram negative	Gram negative	Gram positive	Gram positive
Cellular morphology	Rods	Cocci	Cocci	Diplococci
Motility	Motive	Motive	Non-motive	Non-motive
Indole	+ve	+	-	-
Methyl red	+ve	+	+	-
Vogas Proskauer	-ve	-	+	-ve
Citrate	-ve	-	+	+ve
Urease	-ve	+	+	-
Catalyse			+	-

Table 4. Zone of inhibition of methanolic extract of *Moringa oleifera* against the organisms.

Extract	Zone of inhibition (mm/dm)			
	<i>E. coli</i>	<i>Shigella spp</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>
Methanolic				
10m/ml	-	-	-	-
20mg/ml	-	-	10.0	-
30mg/ml	-	8.0	9.0	-
40mg/ml	-	13.5	15	-
50mg/ml	-	20.0	17	-

Table 5. Zone of Inhibition of aqueous extract of *Moringa oleifera* against the organisms.

<i>Moringa oleifera</i> Leaves	<i>E. coli</i>	<i>Shigella spp</i>	<i>Staphylococcus aureus</i>	<i>Streptococcus pneumoniae</i>
Aqueous Extract				
10m/ml	-	-	-	10.0
20mg/ml	9.0	-	-	13.0
30mg/ml	10.0	-	-	13.0
40mg/ml	10.0	9.0	-	15.0
50mg/ml	10.0	15.0	-	18.0

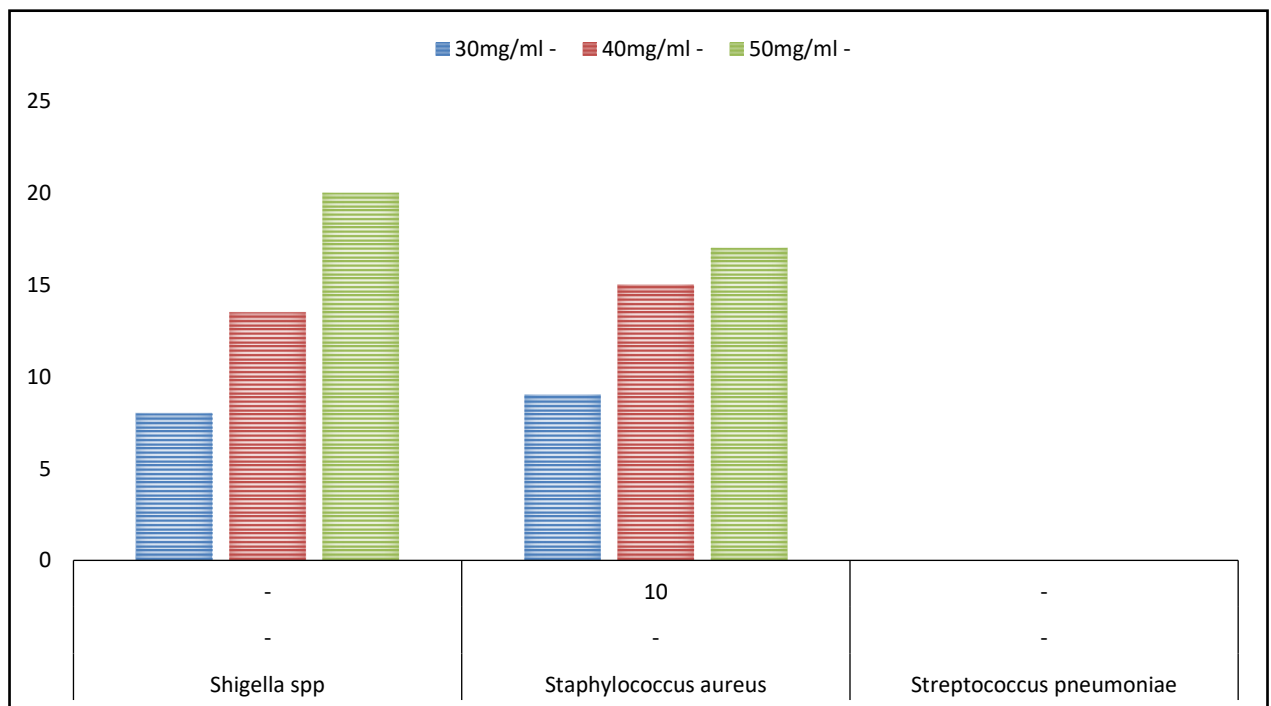
Figure 1. Chart showing zone of inhibition of methanolic extract of *Moringa oleifera* against the organisms.

Figure 2. Chart showing zone of inhibition of aqueous extract of *Moringa oleifera* against the organisms

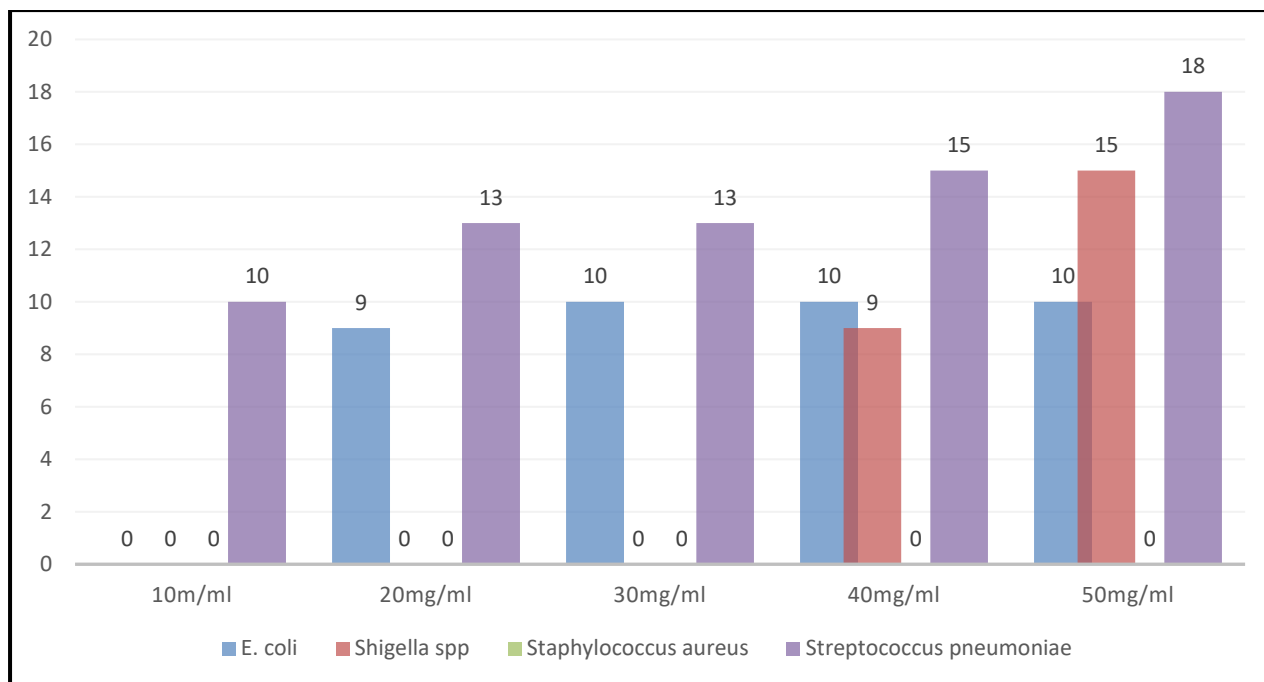


Table 6. The minimum inhibitory concentration of aqueous extracts of *Moringa oleifera* on test organisms

Treatment	C(AQ)E
Test Organism	
EC	0.0240 ^b
SHG	0.2800 ^a
ST	0.005 ^b
STR	0.0576 ^{ab}
S.E	0.1060
Sig.	NS
Conc. Levels (mg)	
10	0.0450 ^a
20	0.0925 ^a
30	0.0925 ^a
40	0.2158 ^a
50	0.0125 ^a
S.E	0.1411
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT). ** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E. coli*, SHG = *Shigella*, ST = *Staphylococcus aureus*, C(AQ)E = Concentration of aqueous methanolic extract.

Table 7. The minimum inhibitory concentration of methanolic extracts of *Moringa oleifera* on test organisms

Treatment	C(me)E
Test Organism	
EC	0.0000 ^a
SHG	0.1200 ^a
ST	0.0540 ^a
STR	0.0000 ^a
S.E	0.0682
Sig.	NS
Conc. Levels (mg)	
10	0.0000 ^a
20	0.1000 ^a
30	0.0000 ^a
40	0.0000 ^a
50	0.0770 ^a
S.E	0.1411
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT). ** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E. coli*, SHG = *Shigella*, ST = *Staphylococcus aureus*, C(me)E = Concentration of aqueous methanolic extract.

Table 8. The minimum inhibitory concentration of aqueous leave extracts of *Moringa oleifera* on test organisms.

Treatment	L(AQ)E
<i>Test Organism</i>	
EC	7.8000 ^b
SHG	4.8000 ^{bc}
ST	0.0000 ^c
STR	15.0000 ^a
S.E	2.6533
Sig.	**
<i>Conc. Levels (mg)</i>	
10	4.5000 ^a
20	6.0000 ^a
30	8.0000 ^a
40	10.000 ^a
50	6.0000 ^a
S.E	5.1672
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT). ** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E. coli*, SHG = Shigella, ST = *Staphylococcus aureus*, L (AQ)E = Concentration of aqueous leave extract

Discussion

The findings of the preliminary phytochemical investigations and the results of antibacterial activity were shown in the respective tables. The preliminary phytochemical tests performed were on to know their presence and from the phytochemical investigations it was observed that alkaloids, tannins, flavonoids, terpenoids, saponins, phytosterols, phenols, steroids and quinones were present in the extracts as well as some were absent. Phytochemical analysis similar to our results by **Maluventhann and Sangu** [23], stated that ethanol, chloroform and aqueous extract of *Cardiospermum halicacabum* leaves showed the presence of flavonoids, tannins, steroids and glycosides. Antibacterial activity of *Cardiospermum halicacabum* was studied by same workers who reported that ethanol extract was active against *Streptococcus aureus* followed by *Salmonella typhi*, *E. coli* & *P. aeruginosa*. It was also related to the present study.

Moreover, the results recorded in **table (2)**, stated that, methanolic and aqueous extract had considerable activity against *Shigella*, which was more

Table 9. The minimum inhibitory concentration of methanolic leave extracts of *Moringa oleifera* on test organisms.

Treatment	L(me)E
<i>Test Organism</i>	
EC	0.0000 ^a
SHG	0.1200 ^a
ST	0.0540 ^a
STR	0.0000 ^a
S.E	0.0682
Sig.	NS
<i>Conc. Levels (mg)</i>	
10	0.0000 ^a
20	0.1000 ^a
30	0.1175 ^a
40	0.0000 ^a
50	0.0000 ^a
S.E	0.0770
Sig.	NS

Means within a column followed by the same letters are statistically not significant at 5% level of probability using Duncan's multiple range test (DMRT)

** = Significant at 1%, * = Significant only at 5% and Ns = Not significant at 5%. EC = *E. coli*, SHG = Shigella, ST = *Staphylococcus aureus*, L(me)E = Concentration of methanolic leave extract.

active than the standard against *Shigella*. Previous study conducted by **Ghebremichael et al.** [24], suggested that the essential oil of *O. majorana* possessed antibacterial activity. The work conducted by **Farooqi and Sreeramu** [25], revealed that the leaves of marjoram had antimicrobial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Salmonella typhi*. Similarly, antimicrobial activity of ethanolic, chloroform and water extract of *Marrubium vulgare*, was further assessed against, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, were recorded [26]. As well as differ from that of **Abdallah and Ali** [21], that reported to have high level of significances.

More so, **Imran et al.** [27], studied the phytochemical analysis of *Azadirachta indica* leaves by using different solvent such as petroleum ether, chloroform, methanol which showed the presence of triterpenes, glycosides and fatty acids. Other phytochemicals studied in this analysis were absent in all extract of leaves. Antibacterial activity of *Azadirachta indica* was analysed by previous workers

showed that the chloroform extract of leaves possesses significant activity than petroleum ether and methanol extracts [27]. Early studies proved ethanol as the most efficient solvent for extracting broad spectrum of antibacterial compounds from plants. Similarly the extract of *Azadirachta indica* is active against *E.coli* followed by *Staphylococcus aureus*. Earlier observation done by **Srinivasan et al.** [28], also showed the antifungal and antibacterial activity of *A. indica*.

Nevertheless, findings of **Panda et al.** [29], who studied the antibacterial activity and phytochemical screening of ethanol; chloroform and extract of *Vitex negundo* were similar to the present findings. Antibacterial activity on *vitex negundo* tested by **Farooqi and Sreeramu, Kumar et al., Ben Gueddeur and Ahmad et al.** [25, 30-32], reported negative results. On the other hand, **Valsaraj et al.** [33], reported positive results against *B. subtilis*, *S. epidermis*, *E. coli* & *P. aeruginosa*. **Bukar et al.** [34], reported that *Moringa oleifera* leaf ethanolic extract had the broadest spectrum of activity on the test bacteria. The results showed that activity against four bacterial isolates *Enterobacter spp* (7 mm), *Staphylococcus aureus* (8mm), *Pseudomonas aeruginosa*, (7mm) and *Escherichia Coli* (7 mm) were sensitive at concentration of 200 mg/ml. while *Shigella spp* and *Salmonella typhi* were not sensitive at all concentrations used. **Napolean et al.** [35], also reported *Enterobacter spp*, *S.aureus*, *P.aeruginosa*, *S.typhi* and *E.coli* to be sensitive to ethanol, chloroform and aqueous extract of *Moringa olifera* leaf at concentration of 200 mg/1. The ethanol, chloroform and aqueous extract of *O. majorana* and *M. vulgare* because of its strong microbicidal property and superiority over commercial purposes, may prove to be an effective herbal protectant against a wide spectrum of pathogenic bacteria and fungi, since herbal microbicides are non-toxic and eco-friendly [23].

Conclusion

Moringa oleifera leaves possessed a very good activity on common medical conditions but few used it for preventing and treating malnutrition. Analysed phytochemicals indicated possible preventive and curative properties of *Moringa oleifera* leaves. There is a need to carry out more pharmacological studies to support the use of *Moringa oleifera* as a medicinal plant.

Conflict of interest

No conflict of interest declared as far this research is concerned.

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