

Phytochemical compositions and antibacterial effects of crude extract of leaf of *Moringa oleifera* on bacteria isolates from well water at Iworoko-Ekiti, Nigeria

ADEFEMI GEORGE OGUNLEYE¹, KEHINDE ADEWOLE OYENIRAN¹, OLUSEGUN ADEDAYO ADEDEJI¹, BOLATITO BOBOYE¹

¹Department of Microbiology, The Federal University of Technology Akure 342052, Ondo State, Nigeria

Received 11 March 2016

Accepted 30 May 2016

Introduction

Diseases caused by microorganisms especially in the tropics are often very severe because of the prevailing warm and humid conditions that promote pathogen growth, enhancing their virulence and thus making such diseases very difficult to manage [1]. The lowly rated socioeconomic statuses of the developing countries also play vital roles in the progression of infections [2]. In Nigeria, the use of medicinal plant to cure different disease conditions is very common as these plants are believed to be rich in several different secondary metabolites also known as phenolics [3]. The potency of such plants is therefore dependent on the abilities of these phenolics to provide a novel based antimicrobial approach that is different from what the pathogen usually encounters, thus extirpating it [4]. Previous studies have shown that microbial resistance to medicinal plants is generally very minimal when compared with standard, constantly used commercial drugs [5]. This could also be explained in terms of novel phytochemical compositions.

From the relics of time, medicinal plants have been cherished as drugs because of their ability to alter biological processes. Economically, these plants are vital hallmarks of folkloric health care especially in West Africa [6]. In multiplicity, plants are believed to be between 250,000 to 500,000 species spread across the globe.

Correspondence to: Mr Kehinde Adewole Oyeniran

Email: oyenirankehinde@gmail.com

ABSTRACT

Objective: Phytochemical compositions of *Moringa oleifera* leaf are believed to be a major contributing factor to its antibacterial activities. The present study investigated the phytochemical compositions and antibacterial effects of crude extract of leaf of *Moringa oleifera* on bacterial isolates from well water at Iworoko-Ekiti, Nigeria

Methods: Phytochemical compositions and antibacterial effects of crude leaf extracts of *M. oleifera* on *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus subtilis*, *Proteus mirabilis* and *Staphylococcus aureus*. (Bacterial isolates from well water) were investigated using the agar diffusion method. Qualitative and quantitative phytochemical analyses were initially accomplished using standard methods.

Results: Phytochemical screenings of the *M. oleifera* leaf ethanol-extracts revealed the presence of phytochemical compounds. Alkaloid, Saponin, Flavonoids, Steroids and Cardiac glycosides were present while Phlobatanins and Anthraquinones were absent. Antibacterial activities of *M. oleifera* increased as the concentration increased. The most susceptible pathogens at the concentration of 125 mg/ml were *Escherichia coli* and *Klebsiella pneumoniae* with zone of inhibitions of 10 ± 0.00 and 10.0 ± 0.58 mm. The minimum inhibitory concentration (MIC) ranged between 25-100mg/m.

Conclusions: The leaf extract of *M. oleifera* is a potent antibacterial agent. Its activity is dose dependent and could be linked to the presence of secondary metabolites.

KEY WORDS:

Antibacterial
Ethanol extracts
Moringa oleifera
Phytochemical
well water

They flourish in virtually all environments from the marshy planes to the deserts, and from halophiles to fresh water and ponds [7]. For easy ordering and identifications, plants are divided into different taxonomical groups known as kingdoms; these are further structured into phylum, class, order, family, genus and species. Within the

family of the Moringaceae is found an amazing multipurpose plant called the Moringa (*Moringa oleifera*) [8].

Moringa oleifera is a tropical plant endowed with a small to medium-sized perennial softwood tree with timber of less economic potentials. It is the most widely cultivated species of a monogeneric genus plant family of *Moringaceae*. Despite being native to sub-Himalayan regions of northern India, the plant as a result of its prized values has been planted around the world and naturalized in many climes [9]. In Nigeria, it is known by many native names such as ‘zogeli’ in Hausa, ‘okweoyibo’ in Igbo, ‘ewe ile’, ‘igi iyaanu’ or ‘eweigbake’ in Yoruba and ‘dogalla’ in Taroh [10]. *Moringa oleifera* has also been dubbed: horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nebeday, saijhan, sajna and ben oil tree [11-12]. Its medicinal properties have been ascribed to different phytochemical compositions of its various parts; the roots, bark, leaf, flowers, fruits, and seeds [13]. As a result of therapeutic attributes of *M. oleifera*, the present study will investigate the antibacterial activities of crude extract of its leaf against pathogenic bacteria isolates from well water at Iworoko-Ekiti metropolis, Ekiti-State, Nigeria.

Materials and Methods

Sample collection and preparation

Plant Sample

Fresh *Moringa oleifera* leaves were obtained from a local Moringa plantation in Ado-Ekiti. The identification and authentication of the plant were accomplished in the Herbarium of the Department of Plant Science, Ekiti State University Ado-Ekiti, Ekiti State. The leaf was collected and dried in a container in the laboratory for a period of five weeks. The dried leaf material was pounded with a wooden mortar and pestle until they became powdery in form. The extract of the leaf was prepared with ethanol. A mass, 200g, of dried powdered leaf was weighed into different conical flasks and was labeled. The 750 ml of ethanol (98%) was added into a conical flask. The extraction was allowed for a period of 3 days after which the mixture was filtered with a muslin cloth. Filtrate obtained was concentrated using a rotary evaporator (model RE-52A Union Laboratories, England) under re-

duced pressure. The concentrate was then stored in the refrigerator until used.

Test organisms

Previously isolated and identified bacterial pathogens from well water samples at Iworoko Ekiti metropolis were sub-cultured and then cultivated in broth. These pathogens are: *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus subtilis*, *Proteus mirabilis* and *Staphylococcus aureus*.

Qualitative Phytochemical Analysis of the Medicinal Plants

Phytochemical analysis for qualitative detection of alkaloids, tannin, saponin, flavonoids and phenol were performed on the extracts as follows:

Test for Saponins

The ability of saponins to produce frothing in aqueous solution was used as a screening test for saponins. About 0.5 g of each plant extract was shaken with distilled water in a test tube, frothing which persisted on warming was taken as evidence for the presence of saponins [14].

Test for Tannins

A weight of 5g of each portion of plant extract was stirred with 100 ml of distilled water, filtered and ferric chloride reagent was added to the filtrate. A blue-black green precipitate indicated the presence of tannins [15].

Test for Alkaloids

A weight of 0.5 g of the extract was diluted with 10 ml of acid alcohol, boiled and filtered. Two milliliters of diluted ammonia were added to 5 ml of the filtrate. Five milliliters of chloroform were added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Meyer's reagent was added to one portion and Dragendorff's reagent to the other. The formation of a cream (with Meyer's reagent) or reddish brown precipitate (with Dragendorff's reagent) was taken as positive for the presence of alkaloid [15].

Test for Flavonoids

A weight of 2 g of powdered sample was detanned with acetone. The sample was placed on a hot water bath for all traces of acetone to evaporate. Boiling distilled water was added to the detanned sample. The mixture was filtered while hot. The filtrate was cooled and 5ml of 20 % sodium hydroxide was added to equal volume of the filtrate. A yellow solution indicates the presence of flavonoids [15-16].

Test for Phlobatannins

Deposition of red precipitate, when aqueous extract of plant was boiled with aqueous HCL acid, was taken on evidence of phlobatannins [17].

Test for Steroids

A volume of 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of the sample with 2 ml tetraoxosulphate VI acid. The change from violet to blue or green indicate the presence of steroid.

Test for terpenoids (hayashi test)

A volume of 5 ml of each extract was mixed in 2ml chloroform and concentrated tetraoxosulphate VI acid was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids [17].

Test for Cardiac Glycosides (Obdoni test)

A 5 ml of the extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below ring, while in the acetic acid layer, a greenish ring from just gradually throughout the thin layer [15].

Quantitative Phytochemical Analysis

The medicinal plants were also quantitatively analyzed for the presence of phytochemicals according to the methods described by [17].

Determination of total Saponin

The saponin content of the sample was determined by double solvent extraction gravimetric method [17]. A weight of 5 g of crude sample was mixed with 50 cm³ of 20 % ethanol and this was extracted 80 cm³ diethyl ether.

The ethanol extract was treated with 10 cm³ of 5% NaOH. The solvent was distilled and the residue dried in an oven to a constant weight.

$$\frac{W_2 - W_1 \times 100}{W_3}$$

W₃

Where W₁ = weight of empty filter paper

W₂ = weight of precipitate

W₃ = weight of crude sample

Determination of Tannin

The presence of tannin was determined by using [18] method. A weight 5 g of crude sample was introduced into a 250 cm³ beaker to which was added 50 cm³ distilled water and mechanically shaken for 1 h. The mixture was filtered into a 1000 cm³ volumetric flask and made up to mark with distilled water. A volume of 5 cm³ filtrate was taken and mixed with 2 cm³ 0.1 M Iron (III) chloride in 0.1 M HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 760 nm. The samples were analyzed in triplicate.

Determination of Flavonoids

The total flavonoid contents in the medicinal plants were determined by the method described by [15]. A weight of 10 g of the plant samples were extracted with 60 ml of 80 % aqueous methanol at room temperature. The whole solution was filtered through whatman filter paper. The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight. The samples were analyzed in triplicate.

$$\frac{W_2 - W_1 \times 100}{W_3}$$

W₃

Where W₁ = weight of empty filter paper

W₂ = weight of precipitate

W₃ = weight of crude sample

Determination of total Alkaloid

The total Alkaloid contents in the medicinal plants were determined using the method described by [19]. A weight of 5 g of crude sample was introduced into a 250 cm³ beaker. To this was added 100 cm³ 10% ethanolic solution of ethanoic acid, covered and allowed to stand for 4 h. This mixture after 4 h was stirred vigorously and filtered. The filtrate was heated in a water bath for 20 min. Concentrated ammonium hydroxide was added in drops to the

extract until the precipitation was complete. The whole solution was allowed to settle and the precipitated was collected. It was thereafter washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed. The difference between the weight of the filter paper before and after filtration became the quantity of alkaloid present in the sample.

$$\frac{W_2 - W_1 \times 100}{W_3}$$

Where W_3

Where W_1 = weight of empty filter paper

W_2 = weight of precipitate

W_3 = weight of crude sample

Determination of total phenols

Total phenols of the medicinal plants were determined by spectrophotometric method [19]. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. Absorbance was measured at 505 nm in the spectrophotometer. The samples were tested in triplicate.

Determination of Glycoside

A weight 5 g of the sample was weighed into a 250 cm³ beaker and 100 cm³ distilled water was added. This was allowed to stand for 3 h and filtered. To 1 cm³ of the filtrate was added 2 cm³ 3, 5-dinitrosalicylic acid and heated in a water bath for 10 min. The mixture was allowed to cool, 10 cm³ distilled water was added and the absorbance performed at 540 nm. Water was used as the blank and served as the standard. The samples were tested in triplicate.

Antibacterial assay of the *Moringa oleifera* leaf extract using agar well diffusion method.

An antibacterial activity of extracts was determined by the agar well diffusion method as described by [20] with slight modification. After standardization using 0.5 Mcfarland Standard, sterile petri dishes were seeded aseptically with 0.1 ml of the 18 hr old broth cultures of the test microorganisms each while about 15 ml of sterilized nutrient

agar was poured aseptically on the seeded plates. The plates were swirled carefully for even distribution and allowed to gel. With the aid of sterile cork borer of 6 mm in diameter, wells were bored on the solidified agar medium. A concentration of 50 mg/ml of the extracts was prepared using 30% Dimethyl sulphoxide (DMSO) as the reconstituting solvent and filter using 0.4µm pores sterilized membrane filter paper. Each extract (0.5 ml) was then pipetted into the wells of appropriately labelled plates and holes. While pouring, run over was avoided and care was taken not to allow the solution to spill to the surface of the medium. The plates were allowed to stand on the laboratory bench for 15 minutes to allow proper inflow of the solution into the medium before incubating the plates in an incubator at 37°C for 24 hrs. The control was prepared by using 0.1 ml of the reconstituting solvent and incubated alongside with the extract. After incubation, zone of inhibition (diameter) formed in the medium was measured in millimeters to determine antibacterial effectiveness of the extracts. The experiment was carried out in triplicates.

Determination of Minimum Inhibitory Concentration (MIC)

Agar well diffusion method of [21] was used to monitor the antimicrobial effect of the different concentrations of the extracts. Concentrations of extracts used in both medicinal plants; *M. oleifera* and *F. exasperata* ranged from 6.25 mg/ml to 125 mg/ml while the control contained a mixture of distilled water and DMSO in ratio 7 to 3 respectively. The minimum inhibitory concentration was obtained by taking the lowest concentration that did not permit any visible growth of the tested organisms.

Statistical Analysis

All experiments were carried out in triplicates and data collected from the study were subjected to analysis of variance. Treatment means were compared using Duncan New Multiple Range test (DNMRT) at 5% level of significance. These analyses were carried out using SPSS version 17.

Results

Qualitative phytochemical screening of the *M. oleifera* leaf extracts have shown the presence of phytochemical compounds as provided on Table 1 below. It was revealed that Alkaloid, Saponin, Flavonoids Steroids and Cardiac glycosides were present while Phlobatanins, Anthraquinones were absent. Quantitative screening has shown in various proportions, the concentrations of the various phytochemical compounds. As shown in Table 2, Alkaloids was the highest as 45 followed by Flavonoids, Glycosides, Saponins, Phenols and Tannins (mg/100ml) at 35.41, 32.51, 25.90, 4.03 and 3.47 respectively. Antibacterial activities of *M. oleifera* (Table 3) increased as the concentration increased. Significant treatment differences

Table 1. Qualitative Analysis of the Phytochemicals in *Moringa oleifera*.

Phytochemical	<i>Moringa oleifera</i>
Alkaloid	+
Saponin	+
Phlobatanin	-
Anthraquinones	-
Flavonoids	+
Steroids	+
Terpenoids	-
Cardiacglycosides	+

+ = Present - = Absent

were conspicuously different between the concentrations.

The most susceptible pathogens at the concentration of 125 mg/mL were *Escherichia coli* and *Klebsiella pneumoniae* with zone of inhibitions of 10 ± 0.00 mm and 10.0 ± 0.58 mm, followed by *Pseudomonas aeruginosa* and *Staphylococcus aureus* with diameter of zone of inhibitions at 9.67 ± 0.58 mm and 8.33 ± 1.53 mm respectively.

The minimum inhibitory concentration (MIC) ranged between 25-100 mg/ml. The extract was effective on *Pseudomonas aeruginosa* at 25 mg/ml making it the lowest with desired suitability. *Bacillus subtilis* was susceptible at 100 mg/ml, while *Staphylococcus aureus* at 50 mg/ml. Other test pathogens were inhibited at 75 mg/ml.

Table 2. Quantitative Analysis of the phytochemicals in *Moringa oleifera*.

Component	<i>Moringa oleifera</i> extract (mg/100ml)
Alkaloids	46.00 ± 0.00^g
Tannins	3.47 ± 0.00^b
Saponin	25.90 ± 0.00^d
Glycosides	32.51 ± 0.10^e
Terpernoids	0.00 ± 0.00^a
Flavonoids	35.41 ± 0.14^f
Steroids	0.00 ± 0.00^a
Phenols	4.03 ± 0.00^c

Values with the same alphabet along the column are not significantly different $P<0.05$.

Table 3. Antibacterial activity of *Moringa oleifera*.

Concentration (mg/ml)	Zones of Inhibition (mm)							
	<i>Pseud. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. typhi</i>	<i>B. subtilis</i>	<i>Prot. mirabilis</i>	<i>Mira-bilis</i>	<i>Staph. aureus</i>
6.25	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
12.5	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
25.0	1.00 ± 0.00^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
50.0	1.30 ± 0.00^b	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	1.33 ± 0.58^b
75.0	3.00 ± 0.00^c	2.33 ± 0.58^b	3.00 ± 0.00^b	2.00 ± 0.00^b	0.00 ± 0.00^a	1.00 ± 0.00^b	3.00 ± 0.00^c	3.00 ± 0.00^c
100.0	4.67 ± 0.58^d	5.00 ± 0.00^c	5.67 ± 0.58^c	4.00 ± 0.00^c	2.67 ± 0.58^b	2.00 ± 0.00^c	6.33 ± 0.57^d	6.33 ± 0.57^d
125.0	9.67 ± 0.58^e	10.00 ± 0.00^d	10.0 ± 0.58^d	6.67 ± 0.58^d	6.00 ± 0.00^c	6.00 ± 0.00^d	8.33 ± 1.53^e	8.33 ± 1.53^e
Control	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a

Values with the same alphabet along the column are not significantly ($P<0.05$) different.

Table 4. Minimum inhibitory concentration of the crude plant extracts.

Bacterial isolate	<i>Moringa oleifera</i> (mg/ml)
<i>Pseudomonas aeruginosa</i>	25
<i>Escherichia coli</i>	75
<i>Klebsiella pneumoniae</i>	75
<i>Salmonella typhi</i>	75
<i>Bacillus subtilis</i>	100
<i>Proteus mirabilis</i>	75
<i>Staphylococcus aureus</i>	50

Discussion

Qualitative phytochemical screening is viable means of finding out the presence of bioactive secondary metabolites of therapeutic importance. Plant leaf and stem have been described by [22] as reserves of bioactive compounds. Terpenoid and Steroids were not quantifiable. The latter is expected as the two compounds are similar in structure and are thus liable to be affected by similar conditions. Phytochemicals present in plants leaves and stem are usually affected by storage and handling during analysis [23]. Conditions such as high temperature and cross reaction between empiric chemical compounds could as well interfere with their recovery [1]. Previous study of [3] had posited in line with the present study that *M. oleifera* leaf is rich in phenolics which are responsible for its various valuable bioactivities. The leave extracts were observably less effective on the pathogen at the lowest concentrations of 6.25 mg/ml and 12.5 mg/ml as there were no visible inhibition zones. This is expected as activities of bioactive compounds may be dose dependent [24]. Mild Inhibition zones were noticed at the concentrations of 25.0 mg/ml and 50 mg/ml which then become clearer at higher concentrations. Antimicrobial activities of any bioactive agent can be evaluated from its ability to inhibit bacterial growth at a given concentration. In the present research antibacterial activities of *M. oleifera* has again corroborated the submissions of [13, 3] that *M. oleifera* is an endowed tree with several health related properties such as antibacterial, antifungal, antihelminthic, antihypertensive, antidiabetic, anticancer etc. The MIC seeks to determine the lowest two-fold concentrations of a given antimicrobial agent that will inhibit the growth of pathogens. The responses of the test organisms in the current

research could be predicated on intrinsic factors such as acquired resistance and physiological genetic endowments. The leaf extract of *M. oleifera* is a potent antibacterial agent. Its activity is dose dependent and could be linked to the presence of secondary metabolites. Minimum inhibitory concentration is relatively effective. *M. oleifera* as food additives may confer a prophylactic protective coverage on people from pathogenic bacterial contaminants in polluted well water.

Conflict of Interest

We declare that we have no conflict of interest.

Acknowledgements

Authors are grateful to the laboratory staff of the Federal University of Technology Akure Ondo state and Ekiti State University Ado-Ekiti. Ekiti state Nigeria.

References

- 1 Akharaiyi FC, Boboye B. Antibacterial and phytochemical evaluation of three medicinal plants. J Nat Prod. 2010;3: 27-34.
- 2 Oyeniran KA. Antagonistic Activities of Microorganisms Associated with Indigenous Black Soap on Some Selected Skin Pathogens. Brit J Pharm Res. 2015;8(2): 1- 7.
- 3 Ademiluyi AO, Oboh G, Aladeselu OH. Effects of Drying Methods on Phenolic Constituents, Antioxidant properties, α -Amylase and α -Glucosidase Inhibitory Properties of Moringa (*Moringa oleifera*) leaf. Proceedings of the 3rd Annual Conference of SoS, FUTA. 2015.
- 4 Boboye B, Alli, J. Cellular effects of garlic (*Allium sativum*) extracts on *Pseudomonas aeruginosa* and *staphylococcus aureus*. Res J Microbiol. 2008; 3(7), 508.513.
- 5 Oladunmoye MK, Adetuyi FC, Akinyosoye FA. Effect of *Cassia hirsuta* (L) extract on DNA profile of some microorganisms. Afri J Biotech. 2006; 8(3):447- 450.
- 6 Akharaiyi FC, Boboye, B. Activities of antibacterial, phytochemical, and antioxidants of *Cnestis ferruginea* DC (connaraceae) extracts against some clinical isolates. Proceedings of the 23rd Annual Conference of the Biotech Society of Nigeria. University of Nigeria, Nsukka, Nigeria. 2010b.
- 7 Aladeselu HO. Effects of Drying Methods on Phenolic Constituents, Antioxidant Properties, α -Amylase and α -Glucosidase Inhibitory Properties of Moringa (*Moringa oleifera*) Leaf - M. Tech Thesis in the Department of Biochemistry, Federal University of Technology Akure, Nigeria. 2015.
- 8 Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. Experimental Assessment of Moringa

- oleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In-vitro and In-vivo Assays. *Evid Based Complement Alternat Med*. 2012; 32: 47-66.
- 9 Martin LP. The Moringa Tree. ECHO Technical Note. North Fort Myers, USA 2007.
 - 10 Fahey JW, Zalcmann AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochem*. 2001; 56(1): 5-51; 59: 237.
 - 11 Olson M. Introduction to the Moringa family. In *The Miracle Tree Moringa oleifera: Natural Nutrition for the Tropics*. Fuglie LL, editor. Dakar: Church World Service. 2001.
 - 12 Rajangam J, Azahakia R, Manavalan S, Thangaraj T, Vijayakumar A, Muthukrishnan N. Status of production and utilisation of Moringa in Southern India. In: *The Miracle Tree/ the Multiple Attributes of Moringa*. Dakar: CWS. Tamil Nadu, India Division of Vegetable Sciences, Horticultural College and Research Institute, TamilNadu Agricultural University. 2001.
 - 13 Kumar PS, Mishra D, Ghosh G, Panda GS. Medicinal uses and pharmacological properties of Moringa oleifera. *Intl J of Phytomed*; 2010; 2: 210-216.
 - 14 Sofowora EA. "Medicinal Plants and Traditional Medicine in Africa". John Wiley and Sons Ltd, New York, 1982; 54-56.
 - 15 Trease GE, Evans MS. "Textbook of Pharmacognosy". 14th Edition. Balliere Tindall, London, 1989.
 - 16 Sofowora EA. "Medicinal Plants and Traditional Medicine in Africa". Spectrum books Ltd, Ibadan, Nigeria. 1983.
 - 17 Harborne JB. *Phytochemical Methods*. "A Guide to Modern Techniques of Plant Analysis". Chapman Hall, New York, 1979.
 - 18 Van-Burden TP, Robinson WC. Formation of Complexes between protein and tannin acid. *J Agric Food Chem*. 1981; 1:77.
 - 19 Harborne JB. *Phytochemical methods*, London. Chapman and Hall, Ltd. 1973.
 - 20 Esimore CO, Adikwu MU, Okonta JM. "Preliminary antimicrobial screening of ethanolic extract from the lichen *Usnea sub floridans* (L)". *J Pharm Res Dev* 1998; 3(2):99-100.
 - 21 Ncube NS, Afolayan AJ, Okoh AI. "Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends". *Afr J Biotechnol*. 2008; 7(12):1797-1806.
 - 22 Shanthakumari S, Mohan V, Britto J. 2008. Nutritional evaluation and elimination of toxic principles in wild yam (*Dioscorea* spp.). *Trop and Subtrop Agroecosystems*. 2008;(8): 319-225.
 - 23 Habtamu FG, Negussie R. Antinutritional Factors in Plant Foods: Potential Health Benefits and Adverse Effects. *Intl J Nutri Food Sci*. 2014;3(4): 284-289.
 - 24 Oladunmoye MK, Adetuyi FC, Akinyosoye FA. Effect of *Cassia hirsuta* (L) extract on DNA profile of some microorganisms. *Afr J Biotechnol*. 2009; 8: 447-450.