AGRICULTURAL AND BIOLOGICAL SCIENCES

ISSN (Print): 24490954
ISSN (Online): 26364972

QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF MORINGA OLEIFERA AND VERNONIA AMYGDALINA

[1]Ajayi A., [1]Ude A. N. and [2]Balogun O. J.

[1]Department of Anatomy, Faculty of Basic Medical Sciences, College of Health Sciences, Kogi State University, Anyigba, PMB 1008, Anyigba, Nigeria.
[2]Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

**Corresponding email: abajayi2003@yahoo.com*

Manuscript received: 07/01/2017 Accepted:09/10/2017 Published: December 2017

ABSTRACT

Plants have been known to be used in the treatment and management of diseases, and this is due to the presence of phytochemicals. Thus, the screening of plants, both quantitatively and qualitatively, has got the interest of researchers. *Moringa oleifera* and *Vernonia amygdalina* are two plants that have very rich application in trado-medicine for various purposes.Thus this study seeks to understand the phytochemical profile when both plants are mixed. Ethanol, lime and aqueous extracts were used, of which lime extract gave the highest percentage yield of 27.3%, while ethanol and aqueous extracts gave 13.6% and 11.2% respectively. The qualitative phytochemical screening showed that only phenols and tannins were detected in all the three extracts.Flavonoids were only detectedin the aqueous extract and alkaloids detected in the ethanolic extract only. The quantitative analysis showed the presence of all the five phytochemical classes in all extracts with the lime extract having the lowest values for all phytochemical classes tested for. The aqueous extract showed a generally good concentration in all five phytochemicals. Saponins, tannins and flavonoids were highest in the aqueous extract of 13.2%, 49.32 mg/mL and 2.10mg/mL respectively. Phenols and alkaloids were highest in the ethanolic extract of 5.48 G.A. Eq. mg/mL and 3.2% respectively. This result showed that the mixture of *Moringaoleifera* and *Vernoniaamygdalina* is a good dietary combination.

Keywords:*Phytochemicals, Moringa oleifera, Vernonia amygdalina, bitter leaf, extracts, herbs*

INTRODUCTION

From time immemorial, man depended on plants as sources of drugs and 75% – 90% of the rural populations of the world have still not emerged from the use of plants as herbal remedy (kong *et al.,* 2003). The phytochemicals present in plants are responsible for their use in medical practice especially, trado-medicine (Aliyu *et al.,* 2011, Sexena *et al.,* 2013). These phytochemicals have various protective functions for the plants, but not nutritional function; while at the same time they have various health benefits for man (Sexena *et al.,* 2013). Phytochemicals include vitamins, terpeniods, phenolic acids, lignins, stibenes, tannins, flavonoids, quinines, coumarines, alkaloids, amines and betalains (Gracelin *et al.,* 2013).

Moringa oleifera (*M. oleifera*) is a plant that naturally grows in the tropics, which include areas like India, Africa, Arabia, South Asia and South America. It belongs to the family called Moringaceae(Vinoth *et al.,* 2012). It is a multifunctional plant as it is used for food, as water cleaning material, and used in biofuel production (Torres-Castillo *et al.,* 2013). The leaves are known to provide Vitamin C, especially when raw, and are also a good source of Vitamin B (Patel *et al.,* 2014). All these and many more makes *M. oleifera* a well-documented plant (Manikandan *et al.,* 2016). The leaves, apart from vitamins also contain essential amino acids, proteins and minerals in high content which makes it a good and ideal nutritional supplement (Elangovan *et al.,* 2014). Due to its high content in phytochemicals, and also other nutrients, trado-medical practitioners have employed it in the treatment and management of various diseases which some include diabetes, arterial tension, stomach ulcers, diarrhea, gastric discomfort, skin infections, etc. (Nweze and Nwafor, 2014). The trado-medical practitioners makes use of every part of the plant (Abalaka *et al.,* 2012,Kasolo *et al.*, 2011, Manikandan *et al.*, 2016), little wonder why some refer to it as "miracle tree" (Vinoth *et al.,* 2012). The plant has been shown to have some pharmacological uses. Various reports have shown that different solvents extracts of the plant, especially the leaves, is a good antibacterial agent (Abalaka *et al.,* 2012, Adline and Devi, 2014, Manikandan *et al.,* 2016,). The focus on the leaves may be due to its high usage as a vegetable in human diet, though other parts of the plant are also rich in nutrients and phytochemicals. The antimicrobial function of the plant has also been reported (Imohiosen *et al.,* 2014, Napolean *et al.,* 2019 Ojiako, 2014). Other pharmacological reports indicate that it has antifungal (Vinoth *et al.,* 2012, Patel *et al.,* 2014) and antioxidant (Elangovan *et al.,* 2014, Torres-Castillo *et al.,* 2013) properties. The various phytochemical analyses and assays carried out on the leaves on M. oleifera indicates that it contains tannins, steroids and terpeniods, flavonoids, saponins, anthraquinones, alkaloids and reducing sugars (Kasolo *et al.,* 2010). There is also the presence of essential and semi-essential amino acids in different amount (Isitua *et al.*, 2015).

Vernonia amygdalina (*V. amygdalina*) commonly called "Bitter Leaf" in English Language (Oguwike *et al.*, 2013) is a member of the Asteraceae family, a small ever green shrub of the tropical African region, especially in West Africa (Ikhajiangbe-Happy *et al.,* 2014). It is known by different names such as Onugbu (Igbo), Shiwaka (Hausa), Ewuro (Yoruba) and Kinologbo (Ijaw) (Kigagha and Onyema, 2015). It is usually used for dietary purposes, especially after washing the leaves to remove the bitter taste (Oboh and Masodje, 2009). The bitter taste is due to factors such as alkaloids, saponins, tannins and glycosides (Adetunji *et al.,* 2013). In ethno-medicinal practice, the roots and leaves are used to treat fever, kidney problems, hiccups and stomach discomfort. Its blood clothing properties and its ability to reduce blood glucose has also been exploited in the trado-medical practice (Udochukwu *et al.*, 2015). Also reported are its uses as treatment of gastro-intestinal problems, malaria, tooth ache and fertility problems (Eyo *et al.,* 2013).Some of its pharmacological activities are antibacterial (Adetunji *et al.,* 2013, Kigagha and Onyema, 2015, Udochukwu *et al.,* 2015), antimicrobial and hypolipaemic and anti-diabetic effect (Ikhajiangbe-Happy *et al., 2*014, Oboh and Masodje, 2009). It is also a good haemostatic control agent and a good blood sugar reducing agent (Oguwike *et al.,* 2013). Proximate analysis of the leaves of *V.* amy*gdalina* shows that it is a good dietary item. The phytochemical analysis of *V. amygdalina* shows that it contains proanthocyanidins, flavonoids, alkaloids and phenols (Momoh *et al.,* 2015). Saponins and hydrochyanide were also found to be present (Evong *et al.,* 2011).

It is evident from the literature that several studies have examined thephytochemical profile of *M. oleifera* and *V. amygdalina* (Evong *et al.,* 2011, Isitua *et al.*, 2015, Kasolo *et al.,* 2010, Momoh *et al.,* 2015). However, these studies only examinedthe plantsindependent of each other. Moreover, as part of efforts in our lab to understand the effect of the combination of these two plants on diabetes and hypertension, we investigate in this study the qualitative and quantitative phytochemical properties of the combination of *M. oleifera* and *V. amygdalina*.

MATERIALS AND METHODS

Collection and Preparation of Plant Leaves and Extracts

M. oleifera and *V. amygdolina* leaves were harvested at night from farm site in Anyigba Community, Kogi State, North Central Nigeria. Specimens were air-dried for three (3) weeks and then pulverized to powder. 90g of ground *V. amygdalina* leaves sample and 150g of ground *M. oleifera* leaves sample mixed together were soaked in 1000ml of ethanol, lime and distilled water (aqueous) for three (3) days. The extracts of the various solutions were then concentrated in-vacuo to give the ethanol, lime and aqueous extracts respectively.

Phytochemical Analysis

Qualitative Analysis

The qualitative phytochemical analysis was done using standardized procedures according to Tiwari *et al* (2011). The extract was subjected to phytochemical screening for alkaloids (Mayer's test and Wagner's test), saponins (Froth test and Foam test), tannins (Gelatin test), flavonoids (Alkaline reagent test and lead acetate test) and phenols.

Alkaloids

0.5 g of the extracts (ethanol, aqueous and lime) were mixed in 8ml of 1% HCL, this was warmed and filtered. 2ml of each the filtrates was treated with Mayer's reagent. The present of alkaloid shows turbidity or precipitate formation.

Tannins

0.5 g of the extracts (ethanol, aqueous and lime) was dissolved in 2 ml of distilled water, 2 drops of diluted ferric chloride solution was added. A dark green or blue green coloration indicates the presence of tannins.

Flavonoids

0.5 g each of the extracts was dissolved in ethanol (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 mins) indicates the presence of flavonoids.

Saponins

0.5g of each extract was added to 5ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

Phenols

10mg extracts were treated with 2 drops of ferric chloride solution. Formation of bluish black colour indicated the presence of phenol.

Quantitative Analysis

The quantitative phytochemical analysis was done according to the method of Yadav and Agarwala(2011) using spectrophotometric method for total phenol content and aluminum colorimetric method for total flavonoid content, Harbone, (1973) method for alkaloid, Obadoni and Ochuko (2001) method for saponins and Van-Burden and Robinson (1981) method for tannins.

Determination of Alkaloids

0.5 g of ethanolic, aqueous and lime extracts were dissolved in 50mls of their respective solvents. Concentrated ammonium hydroxide was added to the extract solutions drop wise until precipitation was completed. The whole solutions were allowed to settle and the precipitates were collected via filtration (using Wattman filter paper no 24). The residues were weighed and the percentages of total alkaloid contents were calculated as;

$$\text{Percentage of total alkaloids (\%)} = \frac{W2 - W1}{Wi} \times 100$$

Where w1= Weight of filter paper
w2= Weight of filter paper + Precipitate
w*i*= Weight sample.

Determination of Total Phenolic Contents in the Plant Extracts

The concentration of phenols in plant extracts was determined using spectrophotometric method as described by Singleton *et al.*,(1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% $NaHCO_3$. Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of $NaHCO_3$. The samples were thereafter incubated in a thermostat at 45°C for 45 mins. The absorbance was determined using spectrophotometer at λmax = 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of garlic acid and the calibration line was construed. Based on the measured absorbance, the concentration of phenols was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of garlic acid equivalent (G.A. Eq. mg/mL).

Determination of the Total Flavonoid (Aluminum Chloride Colorimetric Method)

The total flavonoid content was determined using aluminum chloride colorimetric method according to Nabavi *et al.,*(2008). Quercetin was used to make the standard calibration curve. The standard calibration curve was prepared using quercetin solutions of various concentrations as follow; 0.1, 0.5, 1.0, 2.5 and 5.0mg/ml dissolved in 80% ethanol. The diluted standard quercetin solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After mixing, the solution was incubated for 30 min at room temperature. The absorbance of the reaction mixtures were measured at 415 nm wavelength with Visible spectrophotometer of model 721 (England). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5ml of ethanolic, aqueous, and lime extracts solutions were reacted with aluminum chloride for determination of flavonoid content as described above.

Tannin Determination by Van-Burden and Robinson (1981) Method

500mg of the sample in each case was taken in a plastic bottle, and 50 ml of distilled water was added. Then it was shaken in a mechanical shaker for 1 h, and filtered in a 50 ml volumetric flask made up to the mark. 5 ml of the filtrate was pipetted out into the test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M $K_4Fe(CN)_6$ (potassium ferrocyanide). The absorbance was measured at 120 nm within 10 min

Saponin Determination(Obadoni and Ochuko, 2001) 20g of each ground sample was put into a conical flask and 100 cm^3 of 20% aqueous ethanol was added. Then the flask was heated on a hot water bath for 4 h. with constant stirring at about 55°C. The mixture was then filtered and the residue was again extracted with another 200ml 20% ethanol. The combined extract was reduced to 40 ml on a hot water bath at about 90°C. The concentrate was transferred into a 250 ml separating funnel, added 20 ml diethyl ether in it followed by vigorous shaking. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in oven, weighed and saponin content was calculated as percentage.

RESULTS AND DISCUSSION

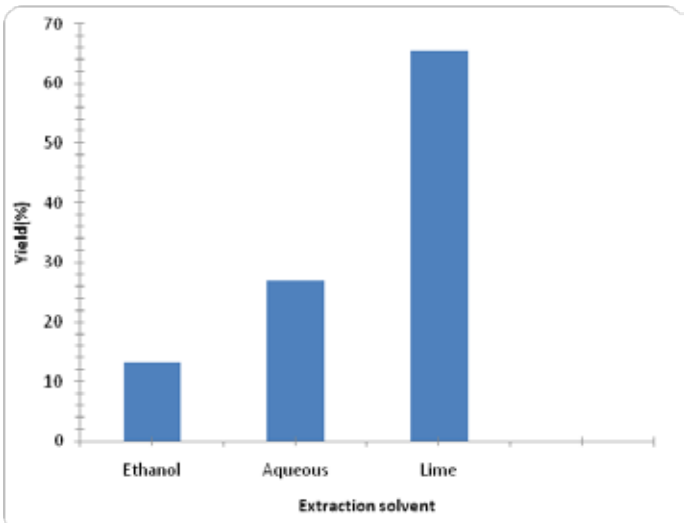

Figure 1: Bar Chart showing extracts yield from each solvent extraction

Table 1: Table showing percentage yield from each solvent extraction

Extract	Ethanol	Aqueous	Lime
Weight of Soaked Sample (g)	240	240	240
Yielded Extract (g)	32.7	27.0	65.6
Percentage Yield (%)	13.6	11.2	27.3

Table 2: Qualitative Phytochemical Screening Analysis of the Mixture of *M. oleifera* and *V. amygdalina*

S/No	Phytochemical	Ethanolic Extract	Lime Extract	Aqueous Extract
1.	Alkaloids	+++	Nil	Nil
2.	Tannins	++	+	+++
3.	Flavonoids	Nil	Nil	+
4.	Saponins	+	Nil	++
5.	Phenols	++	+	++

Table 3: Table showing result of Quantitative Determination of Phytochemicals in the *M. oleifera* and *V. amygdalina* Mixture

Extract	Alkaloids (%)	Phenolics (G.A. Eq. mg/mL)	Flavonoids (mg/mL)	Tannins (mg/mL)	Saponins (%)
Ethanol	**3.2±0.2**	**5.48±1.2**	**1.97±1.1**	**45.59±0.5**	**8.0±1.3**
Aqueous	**2.0±0.4**	**0.89±0.9**	**2.10±0.8**	**49.32±0.4**	**13.2±1.3**
Lime	**1.0±1.0**	**0.25±1.3**	**1.54±0.8**	**44.27±0.8**	**2.6±1.0**

The various solvents extractions showed varied yield with the lime solvent extract having the highest residue of 65.6 g (Figure 1), which corresponds

to 27.3% yield (Table 1). The ethanol extract has a percentage yield of 13.2% (32.7g) while the aqueous extract had a percentage of 11.2% (27.0g). The high pH of lime may be responsible for its ability to extract the high amount observed.

The qualitative phytochemical screening (Table 2) showed that none of the five (5) phytochemical classes tested for were present in all the extract. The five phytochemical classes tested for include alkaloids, tannins, flavonoids, saponins and phenols. Alkaloids were detected highest in the ethanol extract while tannins showed highest detection in the aqueous extract. Flavonoids were not detected in the ethanol extract and also alkaloids were not detected in the aqueous extract. Three of the phytochemical classes, flavonoids, alkaloids and saponins, were not detected in the lime extract. This may be due to the high polarity of the lime which allows polar compounds to be extracted. The less polar ethanol and aqueous extract show better detection to more of the phytochemicals classes tested for.

The quantitative determination (Table 3) shows that tannins have the highest concentration in the three extracts there were expressed in mg/mL, while saponins have the higher percentage content between the saponin and alkaloid determinations that were expressed as percentages.

Alkaloids have been reported to be good in reducing headache and fever due to their analgesic and antibacterial properties (Wadood *et al.,* 2013, Yadav and Agarwala, 2011). Thus, only the ethanolic extract of the sample would be good as an analgesic. In trado-medicine, the use of locally brewed alcohol would also serve in extracting the alkaloids for use. Tannins were found in all the three extracts, but has its highest value in the aqueous extract. Thus, the aqueous extract can be employed in helping in the management and treatment of cancers (Gnanaraja *et al.,* 2014). The high concentration is also in consonance with its ability as a good anticancer agent. Flavonoids were only detected in the aqueous extract and which is also the highest concentration in the quantitative analysis. Thus, the aqueous extract would be good in modifying the body's reaction to allergies, virus and carcinogens (Gnanaraja *et al.*, 2014). Phenols have been known to be good antiaging, anticarcinogen, good in cardiovascular protection etc. (Yadav and Agarwala, 2011). Thus a mixture of bitter leaf and Moringa would be a good dietary item as phenols were present in all the extract. Saponins were detected highest in the aqueous extract and this also was supported by the value in the quantitative analysis of 13.2%. This would be a good antifeeding agent and the aqueous extract would have good detergent properties (Chaieb, 2010).

CONCLUSION

The results show that the mixture of these two plants is good source of phytochemicals. Though the qualitative analysis did not detect some of the phytochemicals in some of the extracts, the quantitative analysis shows that they are present. The result also shows that the phytochemicals are best extracted with water or ethanol for maximum percentage presence of the phytochemicals. The combination of these two plants would serve as a better dietary and medicinal benefit, owing to their synergistic effect.

ACKNOWLEDGEMENT

We acknowledge financial support from Nigeria Tertiary Education Trust Fund (TETFund) and thank Mr. Egwumah Christian for help with the phytochemical analysis as well as for valuable discussions on some part of this project. We also thank the laboratory staff in the Department of Anatomy, Kogi State University, Anyigba for their laboratory assistance.

REFERENCES

Abalaka, M.E., Daniyan, S.Y., Oyeleke, S.B. and Adeyemo, S.O. (2012). Antibacterial Evaluation of *Moringa oleifera* Leaf Extracts on Selected Bacterial Pathogens. *J. Microb Res.2*(2): 1 – 4.

Adetunji, C.O., Olaniyi, O.O. and Ogunkunle, A.T.J. (2013). Bacterial Activity of Crude Extracts of *Vernonia amygdalina* on Clinical Isolates. *J. Microb. Antimicrob5*(6):60–64.

Adline, J. and Devi, A. (2014). A Study on Phytochemical Screening and Antibacterial Activity of *Moringa oleifera*. *Int. J. Res App. Nat. Soc. Sci.2(5)*:169–176.

Aliyu, A.B., Musa, A.M., Abdullahi, M.S., Ibrahim, H. and Oyewale, A.O. (2011). Phytochemical Screening and Antibacterial Activities of Vernoniaambigua, Vernonia blumeoides and Vernonia oocephala. ActaPoloniacPharm. – Drug Res.24(1):67–73.

Chaieb, I. (2010). Saponins as Insecticides: A Review. Tun. *J. Plant Protn, 5*(1):39–50.

Elangovan, M., Dhanarajan, M.S., Rajalakshmi, A., Yayachitra, A., Pardhasaradhi, M. and Bhogireddy, N. (2014). Analysis of Phytochemicals, Antibacterial and Antioxidant Activities of *Moringa oleifera Lam*. Leaf Extract – An In-Vitro Study. *Int. J. Drug Res6*(4):173–180.

Eyo, J.E., Nwachukwu, L.O., Onah, I.E., Atana, C.I., Ekeh, F.N., Ezenwaji, N.E. and Ivoke, N. (2013). Effects of the Aqueous Root Extract of Vernoniaamygdalina on the Haematological Profile of Rattusnorvegicus. *Advan. Life Sci. Tech.11*:19–24.

Eyong, E.U., Agiang, M.A., Atanghwo, I.J., Iwara, I.A., Odey, M.O. and Ebong, P.E. (2011). Phytochemicals and Micronutrients Composition of Root and Stem Bark Extracts of Vernoniaamygdalina Del. *J. Med. Med. Sci, 2*(6):900–903.

Gnanaraja, R., Prakash, V., Peter, S. and Mahendraverman, M. (2014). Qualitative and Quantitative Phytochemical Analysis of Selected Fabaceae Medicinal Plants from Allahabad Region. The Pharm. Inn.3(7):53–56.

Gracelin, D.H.S., John de Britto, A. and Kumar, P.B.J.R. (2013). Qualitative and Quantitative Analysis of Phytochemicals in Five Pteris Species. *Int. J. Pharm. Pharm. Sci. 5*(1):105–107.

Harbone, J.B. (1973). Phytochemical Methods. London Chapman and Hall Ltd. p49–188.

Ikhajiangbe-Happy, I.N., Ezejindu, D.N. and Ezeokafor-Emmanuel, N. (2014). A Comparative Study on the Effect of Vernoniaamygdalina (Bitter Leaf) and Glibenclamide in the Treatment of Diabetes in Alloxan-Induced Diabetic Albino Wistar Rats. *Int. J. Dent. Med. Spec. 1*(1):2–6.

Imohiosen, O., Gurama, H.H. and Lamidi T.B. (2014). Phytochemical and Antimicrobial Studies on *Moringa oleifera* Leaves Extracts. *J. Env. Sci. Tox. Food Tech.8(*1):39–45.

Isitua, C.C., Lozano, M.J.S., Jaramillo, J.C. and Dutan, F. (2015). Phytochemical and Nutritional Properties of Dried Leaf Powder of MoringaoleiferaLam from Machala El Oro Province of Eduador. *Asian J. Plant Sci. Res. 5*(2):8–16.

Kasolo, J.N., Bimenya, G.S., Ojok, L. and Ogwal-Okeng, J.W. (2011). Phytochemical and Acute Toxicity of *Moringa oleifera* roots in Mice. *J. Pharmcg. Phytoth.3*(3):38–42.

Kasolo, J.N., Bimenya, G.S., Ojok, L., Ochieng, J. and Ogwal-Okeng, J.W. (2010). Phytochemicals and Uses of *Moringa oleifera* Leaves in Ugandan Rural Communities. *J. Med Plants Res.4*(9):753–757.

Kigigha, L.T. and Onyema, E. (2015). Antibacterial Activity of Bitter Leaf (Vernoniaamygdalina) Soup on *Staphlococcus aureus* and *Escherichia coli*. *Sky J. Microb. Res.3*(4):41–45.

Kong, J., Goh, N., Chia, L. and Chia, T. (2003). Recent Advances in Traditional Plant Drugs and Orchids. *Acta Pharm.Sinica, 24*(1):7–21.

Manikandan, P., Gnenasekaran, A., julikarthika, P and Prasanth, D.A. (2016). Antibacterial Efficacy of *Moringa oleifera* Leaf against Medically Important Clinical Pathogens. *Int. J. Current Microb. App. Sci.5(4)*:109–116.

Momoh, J., Odetunde, S.K. and Longe, A.O. (2015). Phytochemical Analysis, In-Vitro Evaluation of Antioxidant and Antimicrobial Activity of Methanolic Leaf Extract of Vernoniaamygdalina (Bitter Leaf) Against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Int. J. Current Microb. App. Sci4(5*):411–426.

Nabavi, S.M., Ebrahimzadeh, M.A., Nabavi, S.F. and Jafari, M. (2008). Free Radical Scavenging Activity and Antioxidant Capacity of *Eryngium CaucasicumTrautv* and *Froripia Subpinnata. Pharm. online3*:19–25.

Nepolean, P., Anitha. J. and Renitta, R.E. (2009). Isolation, Analysis and Identification of Phytochemicals of Antimicrobial Activity of Moringa oleifera Lam. *Current Biot.3*(1):33–39.

Nweze, N.O. and Nwafor, F.I. (2014). Phytochemical, Proximate and Mineral Composition of Leaf Extracts of *Moringa oleifera Lam* from Nsukka, South-Eastern Nigeria. *J.Pharm. Biol. Sci.9*(1):99–103.

Obadoni, B.O., Ochuko, P.O. (2001). Phytochemical Studies and Comparative Efficacy of Crude Extract of Some Homostatic Plants in Edo and Delta States of Nigeria. Glob. J. Pure App. Sci.86:203–208.

Oboh, F.O.J. and Masodje, H.I. (2009). Nutritional and Antimicrobial Properties of *Vernonia amygdalina* Leaves. *Int. J. Biomed. Health Sci.5*(2):51–56.

Oguwike, F.N., Offor, C.C., Onubeze, D.P.M. and Nwadoha, A.N. (2013). Evaluation of Activities of Bitter leaf (*Vernonia amygdalina*) Extract on Haemostatic and Biochemical Profile of Induced Male Biabetic Albino Rats. *J. Dent. Med. Sci.11*:60–64.

Ojiako, E.N. (2014). Phytochemical Analysis and Antimicrobial Screening of Moringaoleifera Leaves Extract. *Int. J. Engrn. Sci3*(3):32–35.

Patel, P., Patel, N., Patel, D., Desai, S. and Dhananjay, M. (2014). Phytochemical Analysis and Antifungal Activity of Moringaoleifera. *Int. J. Pharm. Pharm Sci.6*(5):144–147.

Saxena, M., Saxena, J., Nema, R., Singh, D. and Gupta, A. (2013). Photochemistry of Medicinal Plants. J. Pharm. *Phytochem.,1*(6):168–182.

Singleton, V.L., Orthofer, R. and Lamuela-Raventos, R.M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteureagent. Meth. Enzyml, 229:152–178.

Tiwari P., Kumar B., Kaur M., Kaur G. and Kaur H. (2011). Phytochemical Screening and Extraction: A Review. Int. Pharm. Sci.1(1):98–106.

Torres-Castillo, J.A., Sinagawa-García, S.R., Martínez-Ávila, G.C.G., López-Flores, A.B., Sánchez-González, E.I., Aguirre-Arzola, V.E., Torres-Acosta, R.I., Olivares-Sáenz, E., Osorioa-Hernádez, E. and Gutiérrez-Diez, A. (2013). *Moringaoleifera:* Phytochemical Detection, Antioxidants, Enzymes and Antifungal Properties. *Int. J. Exp. Bot.82*:193–202.

Udochukwu, U., Omeje, F.I., Uloma, I.S. and Oseiwe, F.D. (2015). Phytochemical Analysis of Vernonia amygdalina and Ocimumgratissimum Extracts and Their Antibacterial Activity on Some Drug Resistant Bacteria. *Am. J. Res. Comm.3*(5):225–235.

Van-Burden, T.P. and Robinson, W.C. (1981). Formation of Complexes between Protein and Tannin Acid. *J. Agri. Food Chem. 1*:77

Vinoth, R., Asagaperumal R.M. and Balamurugan, S. (2012). Phytochemical Analysis and Antibacterial Activity of Moringaoleifera Lam. Int. J. Res. Biol. Sci.2(3):98–102.

Wadood, A., Ghufran, M., Jamal, S.B., Naeem, M., Khan, A., Shafafar, R. and Asnad (2013). Phytochemical Analysis of of Medicinal Plants Occuring in Local area of Mardan. Biochem. *Anal.Biochem.2(*4):1–4.

Yadav, R.N.S. and Agarwala, M. (2011). Phytochemical Analysis of Some Medicinal Plants. *J. Phyt.,* 3(12):10–14