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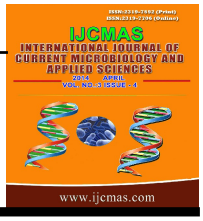

Original Research Article

In vitro antimicrobial activities of *Vernonia amygdalina* on selected clinical isolates

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A B S T R A C T

The antimicrobial activities of *Vernonia amygdalina* ethanol and aqueous leaves extracts were investigated against some selected clinical isolates; *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella spp*., and *Candida albicans*. Agar well diffusion technique was employed to determine the Minimum Inhibitory Concentration (MIC) of the extracts (mg/ml) against the tested clinical isolates. The result showed that *Vernonia amygdalina* leaves extracts possess strong antimicrobial activities against the tested clinical isolates with aqueous leaves extracts exhibiting significantly ($P<0.01$) better zone of inhibition ranging from (11.4 – 12.5mm) against the tested clinical isolates compared to ethanol leaves extracts which showed moderate activities ($P<0.05$) ranging from (10.8 – 12.4mm) at concentration of 100mg/ml, although the differences are not significant ($P>0.05$).The Minimum Inhibitory Concentration (MIC) of the aqueous and ethanol leaves extract for the different clinical isolates both ranged between 12.5 and 50mg/ml. The Phytochemical screening result revealed the presence of flavonoids, anthraquinones, saponins, alkaloids, tannins, cardiac glycosides, steroids, terpenoids and cardenolide, identified in the extracts. The results of this study showed that *Vernonia amygdalina* leaves extracts can be used as potential herbs for drug development for the curing of pathogens tested in this study and these activities could be attributed to the presence of these secondary metabolites.

K e y w o r d s

Antimicrobial activities, *Vernonia amygdalina*, Clinical isolates, Pathogens.

Introduction

Vernonia amygdalina commonly known as bitter leaf (English), Oriwo(Edo), Ewuro (Yoruba), Shuwaka (hausa), and Olubu (igbo), is a tropical shrub that grows up to 3 meters high in the African tropics and other parts of Africa particularly Nigeria, Cameroon, and Zimbabwe. The leaves are dark green coloured with a characteristics odour and a bitter taste. It is reputed to have several

health benefits. It is effective against amoebic dysentery, gastrointestinal disorder and has antimicrobial and anti parasitic activity (Mountdipa *et al.*, 2000). *Vernonia amygdalina* is a perennial herb belonging to the Asteraceae family. The species is indigenous to tropical Africa and is found wild or cultivated all over sub Saharan African (Bosch *et al*., 2005). The leaves are eaten after crushing and washing thoroughly to remove the bitterness (Mayhew and Penny, 1998). However, almost all parts of the plant are pharmacologically useful, both the root and the leaves are used in phyto-medicine to treat fever, hiccups, kidney disease and stomach discomfort among others (Argheore *et al.,* 2000).

Vernonia amygdalina has been ascertained to provide various culinary and medicinal properties, these medicinal properties exert bacteriostatic and bacteriocidal effect on some bacteria (Effraim *et al.,* 2000).

Antihelmitic and Antimalarial properties (Abort and Raserika, 2003) as well as antitumourigenic properties (Izevbigie *et al*., 2004) have also been reported for extracts from the plant. Furthermore, other studies have demonstrated hypoglycemic and hypolipidaemic effect of the leave extract in experimental animals (Nwanjo, 2005).

Many herbalist and native doctors in Africa recommend its aqueous extract for their patients for the treatment of varieties of ailment ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract problems to sexual transmitted diseases and diabetes mellitus among others (Argheore *et al.,* 2000).

Therefore, this study was primarily undertaken to confirm the acclaimed antimicrobial properties of *Vernonia amygdalina* base on their ethno medicinal use in Nigeria.

Materials and Methods

Collection of samples

Fresh leaves of *Vernonia amygdalina* (bitter leaf) free from disease was purchased in a clean polythene bag at Monday market, Maiduguri, Borno state, Nigeria and was transported to the herbarium of the biological sciences department, University of Maiduguri, for identification. The authentication of the plant was done by a botanist. The leaves was washed thoroughly 2-3 times with running tap water and once with sterile distilled water, the leaves material was then air dried on sterile blotter under shade and then was grounded into fine powder using laboratory Pestle and Mortar.

Test organisms

The organisms selected to test the antimicrobial activity of *Vernonia amygdalina* are from different clinical isolates. The organisms are *Escherichia coli*, *Klebsiella spp, Pseudomonas aeroguinosa*, *Staphylococcus aureus, Streptococcus spp*., and Candida *albicans.* The *Escherichia coli*,*Staphylococcus aureus,* Candida *albicans* were isolated from urine samples and *Klebsiella spp, Pseudomonas aeroguinosa* were isolated from wound swab and *Streptococcus spp*. was isolated from throat swab respectively.

The clinical isolates were obtained from the Department of Medical Microbiology and Parasitology, University of Maiduguri Teaching Hospital, Borno state, Nigeria.

All the clinical isolates were sub cultured on Nutrient and MacConkey agar medium respectively for purity and was maintained on nutrient agar slant at 4ºc in the refrigerator until when required for use.

Sample preparation and extraction

10g of dried powder of the plant material was added to 100ml of sterile distilled water or 70%w/v ethanol in order to obtain water or ethanol extracts (100mg/ml). The extraction was done at room temperature for 24 hours for the water extract and 72hours for the ethanol extract (Newton *et al.*, 2002). Muslin cloth was then used to filter the plant residue and the filtrate thus obtained was further purified by filtration through whatman no.1 filter paper (Atata *et al*.,2003).The stock solution of the extract was then sterilized by filtration through Millipore membrane filter of 0.45µm pore size (Ronald, 1995). The sterile extract obtained was then stored in sterile capped and refrigerated at 4ºc until when required for use.

Sterility proofing of the extract

The extract was then tested for sterility after Millipore filtration by introducing 2ml of the sterile extract into 10ml of sterile nutrient broth. This was incubated at 37ºc for 24hours. A sterile extract was indicated by absence of turbidity or clearness of the broth after the incubation period (Ronald, 1995).

Standardization of the bacterial cell suspension

Five colonies of each test organism were picked into sterile test-tube containing sterile nutrient broth and incubated at 37ºc for 24hours. The turbidity produced by this organism was adjusted and used to match the turbidity (opacity) standard prepared as described by Monica (1984).

Determination of minimum inhibitory concentration (MIC) of the extract on the test organisms

The initial concentration of the plant extract (100g/ml) was diluted using double fold serial dilution by transferring 5ml of the sterile plant extract (stock solution) into 5ml of sterile nutrient broth to obtain 50mg/ml concentration. The above process was repeated several times to obtain other dilutions: 25mg/ml, 12.5mg/ml, 6.25mg/ml and finally 3.125mg/ml (Ibekwe *et al.*, 2001). Having obtained the different concentrations of the extract, each concentration was inoculated with 0.1ml of the standardized bacterial cell suspension and incubated at 37ºc for 24hours. The growth of the inoculum in broth is indicated by turbidity or cloudiness of the broth and the lowest concentration of the extract which inhibited the growth of the test organism were taken as the Minimum Inhibitory Concentration (MIC). Negative control was set up as follows: nutrient broth only; nutrient broth and sterile plant extract, and finally positive control containing nutrient broth and a test organism.

Determination of zone of inhibition

Fifteen millimeter (15ml) of sterile nutrient agar was poured into each sterile Petri dish of equal size and allowed to solidify. The surface of this sterile nutrient agar plate was streaked with the pure culture of the standardized bacterial cell suspension. A cork borer (8mm in diameter) was sterilized by flaming and was used to create ditch at the Centre of the plate. The hole so created was then

filled with the plant extract. The plate was allowed to stand for one hour for pre-diffusion of the extract (Esimone *et al.*, 1998) and incubated at 37ºc for 24hours. At the end of the incubation period, the diameter of the zone of inhibition was measured in millimeter using meter rule (Hugo and Russel, 1996).

Phytochemical Analysis of the leaves extracts

The Phytochemical screening of the leaves extracts viz; Flavonoids, Alkaloids, Saponins, Phenol, Glycosides, Volatile Oil, Tannins, Steroids and Terpenoids, Anthraquinone, Cardenolide was analyzed using the method described by Harbourne (1983), Trease and Evan (1989), and Sofowora (1993), respectively.

Test for Flavonoids

Sodium hydroxide method was used for the test. 5g of the sample was weighed and detanned completely with acetone. The residue was extracted in warm water after evaporating the acetone on a water bath. The mixture was filtered and the filtrate was used for the test. 5ml of 10% sodium hydroxide was added to an equal volume of the detanned water extract. A yellow solution indicates the presence of Flavonoids.

Test for Alkaloids

2mls of the extract was measured in a test tube to which picric acid solution was added. The formation of orange colouration indicates the presence of alkaloids.

Test for Saponins

Froth test for saponins was used. 1g of the sample was weighed into a conical flask in which 10ml of sterile distilled water was added and boiled for 5minutes. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and then shaken vigorously for about 30seconds. It was allowed to stand for half an hour. Honey comb froth indicates the presence of Saponins.

Test for Phenol

25ml of extract was added to 2ml of ferric chloride solution, a deep bluish green solution formed indicates the presence of phenol.

Test for Glycosides

25ml of 1ml Sulphuric acid was added to 5ml of the extract in a test tube and boiled for 15minutes, cool and neutralized with 10% sodium hydroxide, and then 5ml of fehling solution A and B was added. A brick red precipitate of reducing sugars indicates the presence of Glycosides.

Test for Tannins

3g of the sample was boiled in 50ml distilled water for 30minutes on a hot plate. The mixture was filtered and a portion of the filtrate was diluted with sterile water in a ratio of 1:4 and 3drops of 10% ferric chloride solution was added. A blue or green colour indicates the presence of tannins.

Test for volatile oils

2ml of extract solution was shaking with 0.1M sodium hydroxide and a small quantity of 0.1M hydrochloric acid. A white precipitate was formed with volatile.

Test for Steroids

Exactly 2ml of acetic anhydride added to 0.5g of the extracts with 2ml of H_2SO_4. The colour changes from violet to blue indicating the presence of steroids.

Test for Terpenoids

About 0.2g extracts was mixed with 2ml Chloroform and 3ml of concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration of the interface formed indicating the presence of terpenoids.

Test for Anthraquinones

0.5g 0f the plant extract was shaked with 10ml of aqueous H_2SO_4 and then filtered while hot, the filtrate was shaked with 5ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution was then added. The presence of violet or red colouration in the ammonical (lower) phase was taken as an indication of combined Anthraquinones.

Test for Cardenolides

Keller-Killiani Test:

0.5g of the plant extract was dissolved in 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was then underlayed with 1ml of concentrated tetraoxosulphate (VI) acid. Appearance of a brown at the interphase showed the presence of digitoxose sugar characteristic of cardenolide.

Statistical analysis

The results obtained from these findings were subjected to ANOVA (Analysis of Variance) by Instant Graph Pad version 3.0 using randomized block design.

Results and Discussion

*Vernonia amygdalina (*bitter leaves*)* is widely consumed in food and used in traditional medicine practice because of its immense medicinal properties which exert bacteriostatic and bacteriocidal effect on some bacteria (Effraim *et al.,* 2000; Okafor *et al*., 2009).

This study revealed that *Vernonia amygdalina (*bitter leaves*)* leaves extract possess strong antimicrobial activities against the tested clinical isolates with aqueous leaves extracts exhibiting significantly ($P<0.01$) better zone of inhibition against the tested clinical isolates compared to ethanol leaves extracts which showed moderate activities ($P<0.05$) at concentration of 100mg/ml, although the differences are not significant ($P>0.05$). This is in conformity with the findings as reported by (Mountdipa, *et al.,* 2000), that the leaves extract of *Vernonia amygdalina* are effective against amoebic dysentery, gastrointestinal disorder and has antimicrobial and antiparasitic activity. The aqueous leaves extract in this study yielded better zone of inhibition (11.4 – 12.5mm) which was found to be more effective against all the tested clinical isolates compared to ethanol leaves extract with zone of inhibition (10.8 – 12.4mm), though the difference was not significant ($P>0.05$). This is also in agreement with the study reported by (Igile, *et al.,* 1995) which revealed that aqueous leaves extract of bitter leaves are used as tonic for the treatment of various illnesses. Several evidences have also shown that herbalist and native doctors in Africa recommend *Vernonia amygdalina* aqueous leaves extract for their patient for the treatment of varieties of ailments ranging from emesis, nausea, diabetes, loss of appetite, dysentery and other gastrointestinal tract

problems to sexual transmitted diseases and diabetes mellitus among others due to its broad spectrum of activities (Argheore, *et al.,* 2000).

In addition, this study contradicts the findings as reported by Obi and Onuaha (2000) and Ogueke *et al* (2006) on their work on *Garcinia kola* extracts that, ethanol is the best solvent for the extraction of most active principle of medicinal properties in plant.

Moreover, the Minimum Inhibitory Concentration (MIC) of the aqueous and ethanol leaves extracts for the different clinical isolates both ranged between 12.5 and 50mg/ml.

Similarly, the aqueous bitter leaves extracts appeared to display higher zones of inhibition on *Escherichia coli, Pseudomonas aeruginosa* with zones of inhibition of 12.5 and 12.2mm respectively followed by *Klebsiella spp* and *Candida albicans* with 11.8mm zones of inhibition, and *Staphylococcus aureus* with the lowest zone of inhibition of 11.4mm. The ethanol bitter leaves extracts also showed significantly ($P<0.05$) wider zone of inhibition on *Candida albicans* 12.4mm compared to aqueous leaves extracts which showed moderate inhibitory effects. This showed that the plant extract have various medicinal values and the inhibitory effects of the leaves extract against the clinical pathogens can introduce the plant as potential herbs for drug development for the treatment of ailments caused by these pathogens(Suleiman,2011).

The Phytochemical Screening of the *Vernonia amygdalina* (bitter leaves) aqueous and ethanol leaves extract was also determined in this study. Phytochemical results of the leaves extracts revealed the presence of flavonoids, alkaloids, anthraquinones, saponins, Tannins, cardiac glycosides, steroids, Terpenoids and Cardenolide. These Phytochemical compounds exert antimicrobial activity through various mechanisms. These secondary metabolites are known to be biologically active and therefore play significant roles in bioactivity of medicinal plants because the medicinal values of medicinal plant lies in these phytochemical compounds which produced a definite and specific action on the human body.

A study conducted by (Oloyede *et al.,* 2011; Boyo *et al*., 2011) on *Vernonia amygdalina* (bitter leaves) revealed that the plant contained flavonoids, saponins, anthraquinones and alkaloids.

Similarly, Imaga *et al* (2013) also detected the presence of alkaloids, tannins, saponins, cardiac glycosides and flavonoids as the most preponderant in their study on aqueous bitter leaf extracts. *Vernonia amygdalina* leaves extracts was also screened to contain Sesquiterpene lactone , flavonoids, Steroids, glycoside e and vernonioside (Igile *et al*., 2000; Izevbigie, 2003; Cimanga *et al*., 2004). Hitherto, secondary metabolites (tannins, saponins, steroids, flavonoids, alkaloids, cardiac glycoside, anthraquinones, terpenoids, etc) exert antimicrobial activity through different mechanism (Igbinosa *et al*., 2009).

Table.1 Antimicrobial activities of *Vernonia amygdalina (bitter leaves) Ethanol leaves extracts on selected clinical isolates*

Zone of Inhibition (mm)	
Test organisms	**Ethanol extracts (mm)**
Escherichia coli	11.3
Pseudomonas aeruginosa	10.8
Klebsiella spp.	11.7
Staphylococcus aureus	11.4
Streptococcus spp.	0.0
Candida albicans	12.4

Table.2 Antimicrobial activities of *Vernonia amygdalina (bitter leaves)* Aqueous *leaves extracts on selected clinical isolates*

Zone of Inhibition (mm)	
Test organisms	**Aqueous extracts (mm)**
Escherichia coli	12.5
Pseudomonas aeruginosa	12.2
Klebsiella spp.	11.8
Staphylococcus aureus	11.4
Streptococcus spp.	0.0
Candida albicans	11.8

Table.3 Minimum Inhibitory Concentration (MIC) of *Vernonia amygdalina (bitter leaves)* Ethanol and Aqueous leaves extracts on selected clinical isolates

Solvents	**Test organisms**				
Mg/ml	*E.coli*	*P.aeruginosa*	*Klebsiella spp.*	*S.aureus*	*C.albicans*
Ethanol extracts	50	12.5	50	25	25
Aqueous extracts	50	25.0	50	12.5	25

Table.4 Phytochemical Screening of *Vernonia amygdalina (bitter Leaves)* Ethanol and Aqueous leaves Extract

Phytochemical Screening Test Test	Ethanol Extracts Results	Aqueous Extracts Results
Anthraquinone	+ +	+ +
Flavonoids	+	+ +
Saponins	+	+
Tannins	+	+
Alkaloids	+	+
Phenol	–	–
Cardiac Glycosides	+	+
Volatile Oil	–	–
Cardenolide	+	+
Steroids	+	+
Terpenoids	+	+

Keys: + + = Abundantly Present
+ = Present in low concentration
- = Absent (not detected)

Flavonoids constituent exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cystostatic and antioxidant properties, anti-cancer activities (Hodek *et al*., 2002; Edeoga *et al*., 2005)

Parehk and Chanda (2007) reported that tannins are known to react with protein to provide the typical tannins effect which is important for the treatment of ulcer (Adegboye *et al*., 2008). Tannins have been found to form irreversible complex with proline-rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis.

Herbs that have tannins as their component are stringent in nature and are used for treating intestinal disorder such as diarrhea and dysentery (Dharmananda, 2003). This observation therefore supports the use of *Vernonia amygdalina* in herbal cure remedies.

Li and Wang (2003) reviewed the biological activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *Vernonia amygdalina* has potential as a source of important bioactive molecule for the treatment and prevention of cancer.

It is possible that steroids occurred as part of aglyconemoietics of other constituents of plant like saponins and alkaloids , steroidal compounds present in *Vernonia amygdalina* extract are of important and interest due to their relationship with various anabolic hormones including sex hormones (Okwu, 2001).

Neumann *et al* (2004) also confirmed the antiviral properties of steroids, Quilan *et al* (2000) worked on steroidal extracts from some medicinal plant which exhibited antibacterial activities on some bacterial isolates.

Alkaloids which are one of the largest groups of phytochemical in plant have amazing effect on human and have led to the development of powerful pain killer medicine (Kam and Liew, 2002). One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms.

In conclusion, this study showed that *Vernonia amygdalina* (bitter leaves) extract exerted significant ($P<0.05$) antimicrobial activities against the tested clinical isolates and might be source of active antimicrobial agents for the development of drugs caused by these pathogens. However, the presence of these phytochemical compounds in *Vernonia amygdalina* (bitter leaves) leaves extract identified in this study could be attributed to the antifungal and antibacterial activities observed.

Recommendations

1. Based on this study, it is recommended that people should develop the habit of eating bitter leaf because it has immense medicinal values in the treatment of various human pathogens.
2. It is also recommended that the plant should be used for the development of drugs for the curing of pathogens tested in this study.
3. It is recommended that further study should be conducted on the mechanisms of action of other parts of the plant viz; root, stem, bark, e.t.c.

References

Abort, A.O., Raserika, B.H 2003. In vivo antimalarial activity of *Vernonia amygdalina. British Journal of Biomedical Science*; 60:89-91 [pubmed].

Adegboye, M.F., Akinpelu, D.A and Okoli, A.I 2008. The bioactive and phytochemical properties of *Garnicia Kola* Hackel seed extract on some pathogens *Afr.J. of Biotechnol.* 721:3934-3938.

Atata, R.F., Sani, A. and Ajewole, S.M 2003. Effect of Stem Bark extracts of *Enantia chloranta* on some Clinical Isolates. *Nig. Society for Experimental Biology*. 15 2: 84-92.

Ademola, I.O., Eloff, J.N 2011. Antihelminthic activity of acetone extract and fractions of *Vernonia amygdalina* against *Haemonchies contortus* eggs and larvae. *Trop. Arum. Health prod*. 432: 521-527.

Adesanoye, O.A., Farombi, E.O 2010. Hepatoprotective effect of *Vernonia amygdalina* astereacea in rats treated with carbontetrachloride, EXP, *Toxicol, pathol* 62:197-206 [pubmed].

Blanco, J.G., Gul, R.R., Bocco, J.L., Meragelman, T.L., Gentiraimondi, S., Flury, A 2001. Aromatase inhibition by an 11, 13-Dihydroderivative of a sesquiterpene lactone .*J. pharmacol, Expt., therap*; 297:1099-1105.

Cimanga, R.K., Tona, L., Mesia, K., Musuamba, C.T., De Bruyne, T.,Apers, S., Hernan, N., Miert ,V.S., Pieters, L., Totte, J., Viletink, A.J 2004. In vitro antiplasmodia activity of extract and fraction of seven medicinal plant used in the democratic republic of Congo .*J. Ethnopharmacol* 93:27-32 [pubmed].

Dharmananda, S. 2003. Allnuts and the uses of tannins in Chinese medicine in proceedings of institutes for traditional medicine port. 3938 land

Oregin. *Afr.J.Biotechnol*.

Esimone, C.O., Adikwu, M.U. and Okonta, J.M 1998. Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usnea subfloridans* L. *Journal of Pharmaceutical Research and Development*. 32: 99-101.

Evans, C. 2003.Interaction between flavonoids and the blood brain barrier: invitro studies. *Neurochemistry*. 85:180-192.

Hugo, W.B. and Russell, A.D 1992. *Pharmaceutical Microbiology*. 5th Ed. Blackwell Scientific Publications Oxford, London. Pp. 258-297.

Harbourne, J.B. 1983. Phytochemical methods. A guide to modern Technique of plant analysis. *Champman and Hall.* London.

Hodek, P., Trefil, P., Stiborova, M 2002. Flavonoids potent and versatile biologically active compounds interacting with cytochrom P450, chemical- *Biol. Intern.* 131:1-21.

Igbinosa, O. O., Igbinosa, E. O. and Aiyegoro, O.A 2009. Antibacterial activity and phytochemical screening of stem bark extract from *Jatropha curcas* Linn. *Afr. J. of pharmacy and pharmacology*. Vol 32:058-062

Ibekwe, V.I., Nnanyere, N.F. and Akujobi, C.O 2001. Studies on Antibacterial activity and phytochemical qualities of extracts of orange peels. *Int. J. Environ Health and Human Development.* 21:41-46.

Izevbigie, E.B., Bryant, J.L., Walker, A. 2004. A novel natural inhibitors of extracellular signal-regulated kinases and human breast cancer cell growth. *Exp Biol Med*. 229:163-169.

Igile, G.O., Oleszek, W., Burda, S., Jurzysta ,M. 1995. Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. *J. Agr. Food Chem*. 43:2162- 2166.

Imaga, N.O.A and Banigbetan, D.O 2013. Invivo biochemical assessment of aqueous extract of *Vernonia amygdalina* bitter leaf. *Intern. Journ. of Nutrition and metabolism.* Vol. 52, Pp.22-27. ISSN 2141-2499, academic journals.

Igile, G.O, Olesezk, W., Burda, S., Jurzysta, M 1995. Nutritional assessment of *vernonia amygdalina* leaves in growing mice. *S. Agric Food chem*.. 43:2162-2166.

Kam, P.C.A., Liew A 2002. Traditional Chinese herbal medicine and anaesthesia. Anaesthesic. 571:1083-1089.

Li, H., Wang; Z., Liu, Y. 2003. Reviewed in the studies on tannins activities of cancer prevention and anticancer. *Zhong-Yaoca*. 266:444-448.

Monica , C. 1984. *Medical Laboratory Manual for Tropical Countries*, Vol. 2. Microbiology. Butterworth and Co Publishers Ltd. Borough Green, Sevenoaks, Kent TN 158 PH. 479Pp.

Nwanjo, H.U. 2005. Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and oxidative status in diabetic rat model. *Nig. J. physiol. Sci.* 201-2:2, 30-42.

Neumann, U.P., Berg, T., Baha, M., Puhi, G., Guckelberger, O., Langreh, J.M., Nuehens, P 2004. Long termoutcome of liver transplant for hepatic C, 10 year follow up. *Transplantation.* 772:226-231.

Newton,S. M., Lau,C.,Gurcha,S.S.,Besra,G.S. and Wright,C. W. 2002. The evaluation of forty-three plant species for invitro antimycobacterial activities; isolation

active constituents from *Psoralea corylifolia* and *Sanguinaria Canadensis*. *Journal of Ethnopharmacology*.79 1:57-67.

Obi,V.I.,Onuaha, C 2000. Extraction and characterization methods of plants and plant product. *In Biological and Agricultural techniques.*

Okwu, D.E. 2001. Evaluation of the chemical composition of medicinal plant belonging to euphorbiacaec. *Pak . Vet .S.* 14:160.

Parekh, J., Chanda, S. 2007. Invitro antibacterial activities of crude methanol extract of wood Fordia Fruticosa Kurtz flower cythacease. *Braz. J. Microbial* 38:2.

Quinlan, M.B., Quinlan, R.J., Nolan, J.M. 2000. Ethnophysiology and herbal treatment of intestinal worms in Dominica west in dies .*J. Ethnopharmacol*. 80:75-83

Ronald, M.A. 1995 *Microorganisms in our World*. Mosby Year Book, Inc. St. Louis. 765pp.

Suleiman, M. N 2011.The in vitro phytochemical investigation on five medicinal plants in Anyigba and its environs, kogi State, Nigeria. *Der. Pharmalia sinica*, 24:108-111. ISSN: 0976-8688. Pelagia Research library.

Shimada, T 2006. Salivary protein as a defense against dietery tannins .*J. chem. Ecol*. 322:1149-1163.

Sofowora, E.A 1993. Medicinal plant and traditional medicine in Africa 2nd edition spectrum book Ibadan Nigeria. Pp. 261-265.

Trease, G.E., Evans, W.C 1989. Text book of pharmacology 12th. Edu. Balleiere, Tinadi London.