Asian Journal of Biological Sciences 5 (2): 105-112, 2012
ISSN 1996-3351 / DOI: 10.3923/ajbs.2012.105.112

Nutritional Evaluation, Phytochemical Screening and Antimicrobial Effects of Aqueous Extract of *Picralima nitida* Peel

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ABSTRACT

The numerous ethno-medicinal applications of *Picralima nitida* plants have called for a high thorough-put investigation of all the parts of the plant including the peel that is usually discarded in order to ensure maximum utilization of the plant. In this study, nutritional evaluation, phytochemical screening and antimicrobial effects of *Picralima nitida* peel were carried out using standard methods, in order to determine the potentials of this discarded part of the plant. The results of proximate contents indicated the following: moisture (49.6%), ash (16.0%), crude fibre (10.5%), crude lipid (7.4%), crude protein (28.4%) and carbohydrate (37.7%) while its calorific value is 265.8 kcal/100 g. Thus, the nutritional value of *Picralima nitida* peel is high and as such it could be used as feed additives. The results of phytochemical screening revealed the presence of flavonoids, saponins, tannins and alkaloids and the aqueous extract had antimicrobial activities against *Escherichia coli* and *staphylococcus aureus* with varying degrees. The most potent inhibitory effect was observed with *Escherichia coli.* These results have revealed that the peel and its extracts have pharmacological active compounds and antibacterial effects and as such could be used in ethno-medicine for the treatment of microbial infection and other ailments.

Key words: *Picralima nitida* peel, nutrient, phytochemical, antimicrobial activity, ethnomedicine

INTRODUCTION

The ethno-medicinal and nutritional use of wild plants are as old as men as they were sources of treatment, food security and income generation (Akubugwo *et al*., 2007a, b; Antia *et al*., 2006; Ifon and Basssir, 1979). These wild plants serve not only as indispensable constituents of human diet but also as important medicinal tools for the treatment of various disease conditions (Aguwu *et al*., 2010; Ogunnowo *et al*., 2010; Suresh *et al*., 2008; Okorondu *et al*., 2006; Edmonds and Chweya, 1997; Shills and Young, 1992). Traditional societies have over the years employed medicinal plants in ethno-medicine for the treatment of various diseases without scientific knowledge of the physiologically active ingredients called phytochemicals which were responsible for the plants' medicinal and pharmacological potentials (Aja *et al*., 2010; Akubugwo *et al*., 2007a; Adimoelja, 2000). Lai (2004) reported the use of plant extracts from *Ocimum gratissimum* and *Azadirachta indica* as a substitute for chemical pesticide for control of Sigatoka disease of banana.

Similarly, Ichor and Ekoja (2011) have shown that the methanolic extract of the plant, *Anogeissus leiocarpus* (Guill and Perr) inhibited the growth of *Salmonella typhi*, *Escherichia coli*

and *Shigella* sp. isolates to varying degrees. Their findings implied that the medicinal and antimicrobial potentials of plants are as a result of their active phytochemicals contents. Their reports which they attributed to the presence of active phytochemicals were consistent with the findings of other researchers (Joseph and Sujatha, 2011; Osadebe and Ukwueze, 2004; Nweze *et al*., 2004; Esimone *et al*., 1998; Ntiejumokwu and Alemika, 1991), who demonstrated the antimicrobial potentials of numerous plants.

Picralima nitida, family Apocynaceae, (common name: Akuamma plant, Igbo: Osi-Igwe) is a species occurring in African forest region, spread through Ivory Coast to Uganda (NNMDA, 2008). *Picralima nitida* bears white flowers (about 3 cm long) with ovoid fruits which at maturity are yellowish in colour. The leaves are broad (3-10 cm) and oblong (6-20 cm long) with tough tiny lateral nerves of about 14 to 24 pairs. The plant has wide varied applications in Nigeria herbal medicine (NNMDA, 2008). It has been shown to possess antiplasmodial, antimicrobial, anti-inflammatory, antipyretic, as well as anti-trypanosomiasis properties. Medicinally, the bark is used to prepare remedies to treat malaria and male sexual impotence, while the fruits are used for dysmenorrhoea and gastrointestinal disorder (Fakoye *et al*., 2000; Ezeamuzieji *et al*., 1994; Iwu and Klayaman, 1992; Ansa-Asamoah and Amposo, 1986; Arens *et al*., 1982).

Numerous works have shown the efficacy of *Picralima nitida* plants extracts against skin conditions of tinea pedis (Athletes foot), *tinea capitis* (ringworm of the head), tinea corporis (ringworm of the body) and *Trypanosoma brucei* (Ezeamuzieji *et al*., 1994; Wosu and Ibe, 1989). Also, the hypoglycemic effects of the bark and seed extracts have been documented (NNMDA, 2008). Several researchers (Fakoye *et al*., 2000; Ezeamuzieji *et al*., 1994; Iwu and Klayaman, 1992) among have reported the medicinal potentials of this plant. However, data on the nutritional potentials and the antibacterial properties of the part of the plant usually discarded as not useful (i.e., the peel) is lacking. This study therefore was designed to assess the nutritive and antibacterial potentials of *Picralima nitida* peel in order to fully harvest its utility.

MATERIALS AND METHODS

Sample collection: The *Picralima nitida* fruits were purchased from Eke-Ukwu market, Owerri, Imo State and identified by the Curator, Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnics, Unwana, Afikpo, Ebonyi State. The *Picralima nitida* fruits were thoroughly washed with tap water and the peels were removed and dried under the sun. The peels were grinded to powder using a mechanical grinder.

Proximate analysis: The recommended methods of the Association of Official Analytical Chemists (AOAC, 1999) were used for the determination of moisture, ash, crude lipid, crude fibre, crude protein and carbohydrate contents.

Estimation of energy value: The sample calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (2.44, 8.37 and 3.57, respectively) used in vegetable analysis (Asibey-Berko and Tayie, 1999).

Phytochemical screening: A portion of the dried and grinded *Picralima nitida* plant sample was subjected to phytochemical screening for the presence of flavonoid, saponins, tannins, glycosides, alkaloid and phenol using the method as outlined in Harborne (1984).

Preparation of aqueous extracts: The *Picralima nitida* peels were extracted by maceration through the addition of exactly 200 g of the powdered sample to 300 mL of distilled water. The container was well covered and shaken vigorously to mix very well and allowed to stand for 5 days with intermittently daily shaking. After the duration, the mixture was then filtered through four (4) fold of sterile cheese cloth to remove the coarse debris. The filtrate was transferred into sterile test tubes and used for antibacterial susceptibility testing.

Test organisms: The studies were performed with *staphylococcus aureus* and *Escherichia coli*. These bacteria were laboratory strains provided by the Microbiology Unit of the Department of Science Laboratory Technology, Akanu Ibiam Federal Polytechnic, Unwana, Ebonyi State, Nigeria. The microorganism were grown in nutrient broth (Biotec, Suffolk, UK) at 37ºC and maintained on nutrient agar (Biotec, Suffolk, UK) slants at 4ºC. The standardized cultures of the organisms were used throughout the experiment.

Preparation and impregnation of *Picralima nitida* disc: Disc of diameter 6.0 mm were punched from a sheet of Whatman No. 3 filter paper (UK) by a perforator and arranged in Petri-dishes allowing a distance of 2-4 mm between each of them. The disc was sterilized in an oven at 160°C for 15 min. The extract of *Picralima nitida* was diluted 4 fold to 0.1, 0.2, 0.4 and undiluted. After the discs were allowed to cool (attaining laboratory temperature), the *Picralima nitida* disc were separately impregnated with 0.1, 0.2, 0.4 and undiluted *Picralima nitida* aqueous extract. The impregnated disc was arranged in separate Petri-dishes and dried by placing them in an incubator at temperature 37°C for 2-3 h.

Bacterial sensitivity test: The level of susceptibility of each of the test organism was determined using agar disc diffusion method as outlined in Suresh *et al*. (2008).

Statistical analysis of data: Analysis of variance (ANOVA) for the data was carried out using SPSS window version 15.1 Chicago, USA and multiple comparison performed using LSD. p-values of <0.05 were considered statistically significant.

RESULT

Proximate composition of *Picralima nitida* peel: The results of proximate composition of *Picralima nitida* Peel is shown in Table 1. The results indicated that *Picralima nitida* Peels contain appreciable amount of nutrients: lipid (7.4%), protein (28.4%) and carbohydrate (37.7%) as well as moisture (10.5%) and Ash (16.0%).

Table 1: Proximate composition of *Picralima nitida* peel

Parameters	Amount (%)
Moisture content	10.5±0.20
Ash content	16.0±0.15
Lipid	7.4±0.08
Protein	28.4±0.16
Carbohydrate	37.7±0.18

Table 2: Phytochemical screening of *Picralima nitida* peel

Phytochemicals	Result
Flavonoids	+
Saponins	+
Tannins	+
Glycosides	+
Alkaloid	+
Phenol	-

Table 3: Level of dilution of extract and mean inhibition diameter (mm)

Test organisms	Level of dilution of extract (mL)	Mean inhibition diameter (mm)
E. coli	0.1	6.00±0.10
	0.2	8.50±0.10
	0.4	12.75±0.01
	Undiluted	2.70±0.10
S. aureus	0.1	3.75±0.01
	0.2	5.50±0.10
	0.4	7.75±0.01
	Undiluted	0.00±0.00

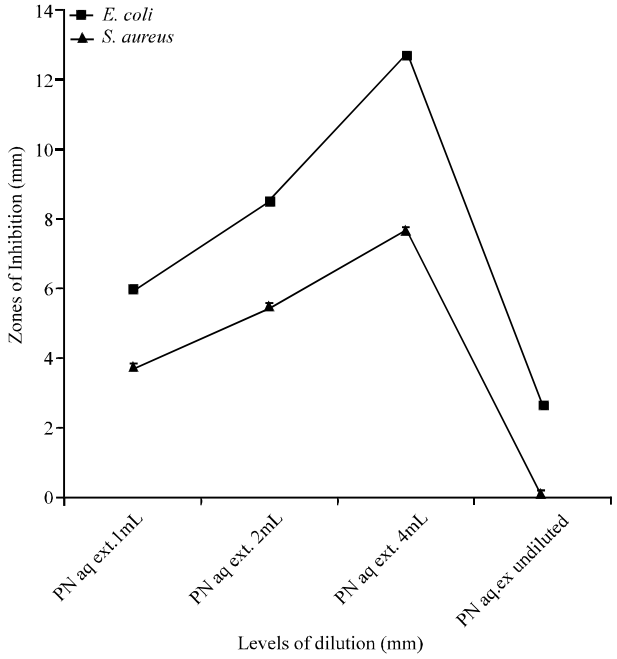

Fig. 1: Variation on the sensitivity of *Picralima nitida* aqueous extract to *E. coli* and *S. aureus* at different levels of dilution

Phytochemical screening of *Picralima nitida* peel: The results of phytochemical screening of *Picralima nitida* peel is shown in the Table 2. The result revealed the presence of flavonoids, saponins, tannins, alkaloids and glycosides while phenols were absent.

Zone of inhibition: The sensitivity pattern of *E. coil* and *S. aureus* on *Picralima nitida* Peel aqueous extract at 0.1, 0.2, 0.4 mL and undiluted is shown in Table 3. Figure 1 shows that the

zone of inhibition of *E. coli* by *Picralima nitida* Peel aqueous extract when diluted at 0.1 mL dilution was significantly lower ($p<0.05$) than the zone of inhibition by extract at 0.2 mL dilution. Also the zone of inhibition of *E. coli* by the extract at 0.4 mL dilution was significantly higher ($p<0.05$) when compared with the zone of inhibition at 0.1-0.2 mL and the undiluted. The zone of inhibition of the *Picralima nitida* Peel aqueous extract to *staph aureus* at 0.4 mL dilution was significantly higher ($p<0.05$) than the zone of inhibition at 0.1 and 0.2 mL. There was no inhibition of *Staph aureus* by the undiluted extract.

DISCUSSION

Proximate and phytochemical analysis is very useful in the evaluation of some bioactive and biological components of fruits and other parts of plants. Evaluation of antimicrobial property of plant is necessary in the discovery of plants with medicinal potentials. This present study assessed the proximate, phytochemical and antimicrobial potentials of *Picralima nitida* peel.

The results (Table 1) indicated that *Picralima nitida* Peels had appreciable amount of nutrients. Similar appreciable nutritional values have been reported by other researchers for similar plants (Aja *et al*., 2010; Akubugwo *et al*., 2007a, b). However, they were variations which could be as a result of plant species, genotype and other environmental factors (Gardens *et al*., 1996; Ezeamuzieji *et al*., 1994). These results have shown that *Picralima nitida* Peels could serve as alternative source of feed for animals and could equally be consumed by man, after quality processing to remove any toxic components.

The results of the phytochemical screening revealed presence of flavonoids, saponins, tannins, alkaloid and glycosides while phenol was found to be absent (Table 2). These findings agreed with earlier reports (Azu and Onyeagba, 2007). However, Iwu and Klayaman (1992) reported absence of glycosides while Ezeamuzieji *et al.* (1994) reported the presence of phenol in their findings. This could possibly be attributed to the differences in the plant species and environmental condition (Fakoye *et al*., 2000). Epidemiological studies have shown that flavonoids intake, are inversely related to mortality from coronary heart diseases and other incidences of heart attacks (Shills and Young, 1992). The invaluable pharmaceutical properties reported for *Picralima nitida* Peel may be attributed to the presence of these bioactive compounds such as flavonoid (Aguwu *et al*., 2010; Adimoelja, 2000; Ezeamuzieji *et al*., 1994).

Our result (Fig. 1) indicated variable zones of inhibition of *Staphylococcus aureus* and *Escherichia coli* by the aqueous extract of *Picralima nitida* disc at different dilutions. The disc diffusion method for antibacterial activity showed significant reduction in bacterial growth in terms of zone of inhibition around the disc. Among bacterial forms tested, the diameter of inhibition zone of the aqueous extract of *Picralima nitida* peel was more sensitive to *Escherichia coli* than *Staphylococcus aureus* (Fig. 1).

The more diluted extract has the largest zone of inhibition, hence the more the extract is diluted the more the active ingredient required to inhibit the microorganism is liberated. Nkere and Iroegbu (2005) observed similar results. That there was no zone of inhibition of *S. aureus* when the extract was not diluted implicated that the bioactive ingredients in the peel is ineffective in the undiluted form. Similar results have been reported by several other researchers working independently on numerous other plants extract (Ichor and Ekoja, 2011; El-Shemy *et al*., 2007; Zumbes *et al*., 2007; Nascimento *et al*., 2000; Singleton, 1999).

Larger zone of inhibition was observed in *E. coli* than *Staph aureus*. This agrees with the work of Nkere and Iroegbu (2005) who reported inhibition zone of 10±1, for *E. coli* and 8±1.75, for *Staph aureus* by aqueous extract of *Picralima nitida* peel. The differences in the zone of inhibition

may be directly related to the susceptibility of test organism to the *Picralima nitida* peel extracts. The factors responsible for the high susceptibility of *E. coli* than *Staph aureus* may be attributed to the differences in the organisms physiology and anatomy and the presence of secondary metabolites (Omogbai and Eze, 2011; Azu and Onyeagba, 2007; Iwu *et al*., 1992). The inhibition of the *Picralima nitida* peel extracts on *S. typhi* and *E. coli*. is of great importance to the health care system since it can be used as an alternative to orthodox antibiotics in the treatment of infections due to these isolate especially as they are becoming resistant to known antibiotics (El-Shemy *et al*., 2007; Nascimento *et al*., 2000; Singleton, 1999).

The antimicrobial activity of plant extracts is, possibly, due to their ability to complex with extra cellular and soluble proteins and to complex with bacterial cell walls disrupting microbial membranes (Neuwinger, 2000; Burkill, 1985). Therefore, the level of inhibition increased as the dilution increases. This could be due to increase in the liberation of the bioactive component by the solvent (Nkere and Iroegbu, 2005). This observation supports the hypothesis that the inhibition involves phenolic compounds which the results revealed is an integral component of *Picralima nitida* peel extracts, because phenolic compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital intracellular constituents (Joseph and Sujatha, 2011; Fu *et al*., 2007; Betoni *et al*., 2006; Li *et al*., 2005; Lopez *et al*., 2005; Arora and Kaur, 1999). Our observation in the present study supports other findings that *Picralima nitida* peels have nutritional values and antimicrobial properties and as such this *Picralima nitida* peel extracts promise to reduce the cost of obtaining health care since the plant is readily available and the cost of preparation is relatively cheaper.

CONCLUSION

Picralima nitida is nutritionally potent and can be used in the formulation of animal feeds. *Picralima nitida* peel aqueous extracts have antibacterial activities against the test bacteria used in this study. Susceptibility of the bacterial varied with the concentration of extract and the organism involved. Phytochemical screening shows that the sample contains useful bioactive components (saponins, tannins, alkaloids) that contributed to antibacterial properties and hence its widely acclaimed medicinal values.

REFERENCES

AOAC, 1999. Methods of Analysis of Association of Official Analytical Chemists. 16th Edn., AOAC, Washington, D.C., pp: 600-792.

Adimoelja, A., 2000. Phytochemicals and the breakthrough of traditional herbs in the management of sexual dysfunction. Int. J. Androl., 23: 82-84.

Aguwu, C.N., C.V. Nkwe, S.I. Inya-Agha and J.M. Okonta, 2010. Antidiabetic effect of *Picralima nitida* aqueous extracts in experimental rabbit model. J. Nat. Remedies, 1: 135-139.

Aja, P.M., A.N.C. Okaka, P.N. Onu, U. Ibiam and A.J. Urako, 2010. Phytochemical composition of *Talinum triangulare* (water leaf) leaves. Pak. J. Nutr., 9: 527-530.

Akubugwo, E.I., N.A. Obasi, G.C. Chinyere and A.E. Ugbogu, 2007a. Mineral and phytochemical contents in leaves of *Amaranthus hybridus* and *Solanum nigrum* L. subjected to different processing methods. Afr. J. Biochem. Res., 2: 40-44.

Akubugwo, I.E., A.N. Obasi and S.C. Ginika, 2007b. Nutritional potential of the leaves and seeds of black nightshade-*Solanum nigrum* L. var. *virginicum* from Afikpo-Nigeria. Pak. J. Nutr., 6: 323-326.

Ansa-Asamoah, R. and A.A. Amposo, 1986. Analgesic effect of crude extracts of *Picralima nitida* seeds. Afr. J. Pharmacol., 1: 35-38.

Antia, B.S., E.J. Akpan, P.A. Okon and I.U. Umoren, 2006. Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. Pak. J. Nutr., 5: 166-168.

Arens, H., H.O. Borbe, B. Ulbrich and J. Stoeckigt, 1982. Detection of pericine a new central nervous system active indole alkaloid from *Picralima nitida* cell suspension culture by opiate receptor binding studies. Plnta Medica, 46: 210-214.

Arora, D.S. and J. Kaur, 1999. Antimicrobial activity of spices. Int. J. Antimicrob. Agents, 12: 257-262.

Asibey-Berko, E. and F.A.K. Tayie, 1999. Proximate analysis of some under-utilized Ghanaian vegetables. Ghana J. Sci., 39: 91-96.

Azu, N.C. and R.A. Onyeagba, 2007. Antimicrobial Properties of Extract of *Allium cepa* (Onions) and *Zingiber officinale* (Ginger) on *Escherichia Coli, Salmonella typhi* and *Bacclus Subtilis*. Int. J. Trop. Med., 3: 123-127.

Betoni, J.E.C., R.P. Mantovani, L.N., Barbosa, L.C. Di Stasi and A.F. Junior, 2006. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Mem. Inst. Oswaldo Cruz, Rio de Janeiro, 101: 387-390.

Burkill, H.M., 1985. Useful Plants of West Tropical Africa. 2nd Edn., Vol. 1, Families A-D, Royal Botanic Gardens, Kew, England, Pages: 960.

Edmonds, J.M. and J.A. Chweya, 1997. Promoting the Conservation and Use of Under-utilized and Neglected Crops. In: Black Nightshade (*Solanum nigrum* L.) and Related Species. Edmonds, J.M. and J.A. Chweya (Eds.). International plant Genetic Resources Institute, Rome, Italy, pp: 73-87.

El-Shemy, H.A., A.M. Aboul-Enein, K.M. Aboul-Enein and K. Fujita, 2007. Willow leaves extracts contain anti-tumor agents effective against three cell types. PLoS One, 2: e178-e178.

Esimone, C.O., M.U. Adujwym and J.M. Okonta, 1998. Preliminary antimicrobial screening of the ethanolic extract from the Lichen *Usnea subfloridans* (L). J. Pharm. Res. Dev., 3: 99-102.

Ezeamuzieji, C., M.C. Ojimaka, E.O. Uzogara and S.E. Oji, 1994. Anti-inflammatory, Anti-phyretic and Anti-Malaria activities of a West African Medical Plant- *Picralima nitida*. Afr. J. Med. Med. Sci., 23: 85-90.

Fakoye, T.O., O.A. Hiola and H.A. Odelola, 2000. Evaluation of the Antimicrobial Property of the stem bark of *Picralima nitida* (Apolnaccae). Phototherapy Res., 14: 368-370.

Fu, Y., Y. Zu, L. Chen, X. Shi, Z. Wang, S. Sun and T. Efferth, 2007. Antimicrobial activity of clove and rosemary essential oils alone and in combination. Phytother. Res., 21: 989-994.

Gardens, A.D., J.R.W. Menzies, A. Macdonald, S.J. Paterson and M. Dowagiac, 1996. The opined activity of hcuammine, akauammicine and akalammidine alkaloids from *Picralima nitida* (from Apocynaceae). Br. J. Pharmacol., 119: 334-339.

Harborne, J.B., 1984. Phytochemical Methods a Guide to Modern Technique of Plant Analysis. 2nd Edn., Chapman and Hall, London, pp: 282-311.

Ichor, T. and E.E. Ekoja, 2011. Antimicrobial properties of methanolic extracts of *Anogeissus leiocarpus* (Guill and Perr). Asian J. Biol. Sci., 4: 570-574.

Ifon, E.T. and O. Basssir, 1979. The nutritive value of some Nigeria green leafy vegetables (Part 2): Distribution of protein, Carbohydrate and Fat. J. Food Chem., 5: 231-235.

Iwu, M.M. and D.L. Klayaman, 1992. Evaluation of the *in vitro* antimalarial activity of *Picralima nitida* extracts. J. Ethnopharmacol., 36: 133-135.

Iwu, M.M., J.E. Jackson, J.D. Tally and D.L. Klayman, 1992. Evaluation of plant extracts for anti-leshmanoial activity using a mechanism-based Radiorespiraometricrote-technique (RAM). Plant. Med., 58: 436-441.

Joseph, B. and S. Sujatha, 2011. Bioactive compounds and its autochthonous microbial activities of extract and clove oil (*Syzygium aromaticum* L.) on some food borne pathogens. Asian J. Biol. Sci., 4: 35-43.

Lai, P.K. and J. Roy 2004. Antimicrobial and chemo preventive properties of herbs and spices. Curr. Med. Chem., 11: 1451-1460.

Li, Y., C. Xu, Q. Zhang, J.Y. Liu and R.X. Tan, 2005. *In vitro* anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. J. Ethnopharmacol., 98: 329-333.

Lopez, P., C. Sanchez, R. Batlle and C. Nerin, 2005. Solid- and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungi strains. J. Agric. Food Chem., 53: 6939-6946.

NNMDA, 2008. Medicinal Plants of Nigeria, South-East Zone. Vol. 1, Nigeria Natural Medicine Development Agency, Federal Ministry of Science and Technology, Lagos, Nigeria, pp: 8-159.

Nascimento, G.G.F., J. Locatelli, P.C. Freitas and G.L. Silva, 2000. Antibacterial activity of plants extracts and phytochemical on antibiotic-resistant bacteria. Braz. J. Microbiol., 31: 247-256.

Neuwinger, H.D., 2000. African Traditional Medicine: A Dictionary of Plant use and Applications. Medpharm Scientific, Stuttgart, Germany, pp: 589-612.

Nkere, C.K. and C.U. Iroegbu, 2005. Antimicrobial Screening of the Root, Seed and Stem bark extracts of *Picralima nitida*. Afr. J. Biotechnol., 4: 522-526.

Ntiejumokwu, S. and T.O.E. Alemika, 1991. Antimicrobial and photochemical investigation of the stem bark of *Boswellia dedziella*. West Afr. J. Pharmacol. Drug Res., 10: 100-104.

Nweze, E.I., J.I. Okafor and O. Njoku, 2004. Antimicrobial activities of methanolic extracts of *Trema guineensis* (Schumm and Thorn) and *Morinda lucida* Benth used in Nigerian Herbal Medicinal practice. J. Biol. Res. Biotechnol., 2: 39-46.

Ogunnowo, A.A., C.N. Ezekiel, C.P. Anokwuru, Y.A. Ogunshola and O.O. Kuloyo, 2010. Anticandidal and antistaphylococcal activity of soap fortified with *Ocimum gratissimum* extract. J. Applied Sci., 10: 2121-2126.

Okorondu, S.I., W. Braide, J.N. Ogbuile and C.O. Akujobi, 2006. Antimicrobial and phytochemical properties of some traditional spices. Nig. J. Microbiol., 20: 1301-1308.

Omogbai, B.A. and F.A. Eze, 2011. Phytochemical screening and susceptibility Of bacteria pathogens to extracts of *Evolvulus alsinoides*. Sci. World J., 6: 5-8.

Osadebe, P.O. and S.E. Ukwueze, 2004. A comparative study of the phytochemical and anti-microbial properties of the Eastern *Nigeria* species of African Mistletoe (*Loranthus micranthus*) sourced from different host trees. J. Biol. Res. Biotechnol., 2: 18-23.

Shills, M.Y.G. and V.R. Young, 1992. Modern Nutrition in Health and Disease. In: Nutrition, Nieman, D.C., D.E. Butter Worth and C.N. Nieman (Eds.). WMC Brown Publishers, Dubugue, USA., pp: 276-282.

Singleton, P., 1999. Bacteria in Biology, Biotechnology and Medicine. 4th Edn., John Wiley and Sons Ltd., Chichester, USA, pp: 66-73.

Suresh, K., B.S. Saravana and R. Harisranry, 2008. Studies on *in Vitro* antimicrobial activity of ethanol extract of *Rauvolfia tetraphylla*. Ethobotanical Leaflets, 12: 586-590.

Wosu, L.O. and C.C. Ibe, 1989. Use of extract of *Picralima nitida* bark in the treatment of experimental trypanosomiasis: A preliminary study. J. Ethnopharmacol., 25: 263-268.

Zumbes, H.J., T.O. Belenu and F.C. Onwuliri, 2007. *In vitro* antibacterial activity of *Anogeissus leiocarpus* leaf extracts on some bacteria associated with diarrhea. Int. J. Natl. Applies Sci., 3: 53-56.