PHYTOCHEMICAL QUANTIFICATION AND *IN VITRO* ANTIOXIDANT ACTIVITY OF THE LEAVES, STEM-BARK AND ROOT OF *Nauclealatifolia*SMITH

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Abstract: The phytochemical composition and anti-oxidant activities using Di-phenyl-1-picryl-hydrazyl (DPPH) Radical Scavenging assay, Ferric Reducing and Antioxidant Power(FRAP) and the Total Phenolic contents (TPC) of the extracts from the leaves, stem-bark and root of *Nauclealatifolia*.The phytochemical analysis revealed the presence of bioactive compounds in the entire plant samples. The leaves of *Nauclealatifolia*contained tannins 0.17 g/100g, alkaloid 2.29 g/100g, 0.83 g/100g flavonoid and 0.56% saponin. The stem bark also contained 0.53, 2.58, 0.08 g/100g and 0.84% while the composition of the root are 0.13, 0.88, 0.32 g/100g and 0.72% tannins, alkaloids, flavonoids and saponin, respectively. The stem bark had the highest antioxidant potential; DPPH (30.60 mg/mL), FRAP (184.64 mg/GAE/g) and TPC (30.16 g/100g). Our findings provide evidence that the extract of *Nauclealatifolia* could be a potential source of natural antioxidants that may be used to combat stress related conditions.The phytochemical analysis supports the extensive use of the leaves bark and root of *Nauclealatifolia*in ethno-medicine in many parts of Africa. Overall, the result suggests that *Nauclealatifolia* could be a potential source of pharmacologically active natural product and/or for development of neutraceuticals.

Keywords: Antioxidants,*Nauclealatifolia*, neutraceuticals, phytochemicals, reactive oxygen species

Introduction

Free radicals, which belong to a group of reactive oxygen species (ROS), are produced through endogenous source, that is, the human body itself, and exogenous sources such as tobacco smoke, burning of fossil fuels and ozone (Krovánkoyá*et al*., 2012). The imbalance between the production of ROS and the activity of the antioxidant defences is referred to as oxidative stress (Škrovánková*et al*., 2012). The inhibiting or protective effects of herbs or spices against the harmful consequences of oxidative stress are due to the presence of natural antioxidants in them (Khalaf*et al*.,2008). Antioxidant based drug formulations are used in the prevention and management of complex diseases which include atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer (Khalaf*et al*., 2008; Devasagayam*et al*., 2004).

Nauclealatifolia Smith (syn: Sarcocephaluslatifolius (Sm.) Gruce) (family: Rubiaceae) is a straggling shrub or small tree of about 4 m high abundantly spread in all inter-tropical Africa. It normally produces interesting flowers, edible, but not appealing, large red ball fruits with long projecting stamens. Commonly used parts of *Nauclealatifolia*include the leaves, roots, stem, and fruits. The fruits serve as key source of food for the baboons, livestock, reptiles, birds, and man (Ayelesoet *al*., 2014; James *et al*., 2011; Faleye and Akinwunmi, 2016). Nkafamiya*et al*. (2006) pointed out that the fruits of *Nauclealatifolia*contain copper, iron, cobalt, calcium, magnesium, zinc, phosphorus, and vitamins (A, B1, B2, C, and E). In Cameroon, the roots are used to treat jaundice, yellow fever, rheumatism, abdominal pains and hepatitis and the bark to treat jaundice and loss of appetite (Ayelesoet *al*., 2014;Faleye and Akinwunmi, 2016). In Nigeria, the stem bark and roots of the plant are used against fever, jaundice, malaria, diarrhea, dysentery, hypertension and diabetes (Ayelesoet *al*., 2014, Faleye and Akinwunmi, 2016). Pharmacological studies of *N. latifolia* have shown antibacterial, antidiabetic and antiplasmodial activities (Donalisioet *al*., 2013; Ayelesoet *al*., 2014). Previous phytochemical studies on *N. latifolia* have yielded a great number of indole alkaloids, triterpenes, steroids and saponins (Donalisioet *al*., 2013). The present study was undertaken to test the ethanol/water (1:1) extract of *N. latifolia* leaves, stem bark and root for its antioxidant activity.

Materials and Methods

Sample collection

The various plant parts from *N. latifolia* were collected in the premises of the Ekiti State University Ado-Ekiti, Ekiti State, Nigeria. The plant was identified and authenticated at the herbarium of the Department of Plant Science, Ekiti State University Ado-Ekiti by Mr. Femi Omotayo.

Sample preparation

The leaves, root, and stem bark were washed with distilled water and air-dried at room temperature for 2 weeks. They were pulverized using a mechanical grinder. The powdered plant material was extracted with 50% ethanol-water; the extracts were filtered and evaporated to dryness with the aid of rotatory evaporator at 50^{o}C. The concentrated extracts were stored in an air tight sample vials pending analysis.

Phytochemical estimation

Alkaloid content was quantified by the gravimetric method (Harborne, 1973); saponin, by combined solvent extraction (Obadoni and Ochuko, 2001) and flavonoids, as described by Böhm and Kocipai-Abyazan (1994) and the tannin content was quantified by the method described by Pearson, (1976).

Evaluation of in vitro antioxidant activity

Estimation of total phenolic compounds (TPC)

Total soluble phenolic content in each plant extract was determined using the Folin-Ciocalteu reagent (FCR) according to the method described by Ojong*et al*. (2016). Briefly, 0.1 mL of each concentration of plant extract was transferred to 100 mL Erlenmeyer flask then final volume was adjusted to 46 mL by addition of distilled water. After 3 min, 1 mL of FCR and 3 mL of Na_2CO_3 (2%) were added to this mixture. The mixture was then incubated for 2 h at room temperature (25°C) and the absorbance was measured at 760 nm. All the tests were performed in triplicate and the results averaged. The concentration of total phenolic compounds in each extract was estimated as milligram of gallic acid equivalent by linear interpolation of a gallic acid standard curve (Vinson *et al*., 1995).

Ferric reducing and antioxidant power (FRAP) assay

The total antioxidant potential of *N. latifolia* was determined using ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996; Ojong*et al*., 2016). FRAP reagent was freshly prepared and mixed in the proportion of 10:1:1 (v:v:v)

for solutions A:B:C, where Ais 300 mmol/L sodium acetate trihydrate in glacial acetic acid buffer (pH 3.6); Bis 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ) (10 mM in 400 mM of HCl), and C= ferric chloride (20 mM). Gallic acid was used for a standard curve with all solutions. Each extract (75 µL) was transferred to a cuvette containing 2 mL of FRAP solution and after agitation, the absorbance was read after 12 min of incubation at a predetermined wavelength of 593 nm. The ferric reducing antioxidant power in each extract was determined as milligram of gallic acid equivalent by linear interpolation of a gallic acid standard curve.

Di-phenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity assay

This spectrophotometric assay used the stable DPPH radical as the reagent to determine the DPPH scavenging activity using the method described by Nyaa*et al.* (2009). 20 µL of the aqueous plant extract was introduced to 2 mL methanol solution of DPPH (0.3 mM) and incubated at 37°C in the dark for 30 min. The extract was replaced by methanol for the control and catechin for the standard. Absorbance of the resulting solution was measured at 517 nm using a spectrophotometer. The percentage DPPH radical scavenging activity was calculated by comparing the results of the test with those of the control using the following equation:

Inhibition % = [(A blank – A sample) / A blank] × 100

Where: A blank is the absorbance of the blank solution (containing all of the reagents except the test compound) and A sample is the absorbance of the test samples. All the tests were performed in triplicate and the results averaged. Ascorbic acid was used as standard.

Results and Discussion

Natural products derived from plant sources have assumed greater importance in recent days, due to the tremendous potential they offer in formulating new drugs which may protect humankind against many diseases (Balunas and Kinghorn, 2005; Khalid *et al*., 2013). The phytochemical and antioxidant contents of medicinal plants may contribute to protection against diseases (Saeed*et al*., 2012). Natural antioxidants have attracted a great deal of attention because of their health-promoting effects (Anwar *et al*., 2006). Oxidative stress occurs when the formation of bioactive oxidative products such as oxidizing agents, free radicals and reactive oxygen species, greatly overwhelms the capacity of the endogenous cellular antioxidant defense system, thus leading to potential damage of the cells and organs, and to the progression of degenerative diseases in humans (Schrader and Fahimi, 2006; Basu, 2010). Attention has been on antioxidant agents of natural origin due to their abilities to scavenge free radicals. Antioxidant capacity is associated with compounds that can protect a biological system against the damaging effect of ROS and reactive nitrogen species (RNS) (Ayeleso*et al*., 2014). The water : ethanol (50:50) extract obtained from different parts (leaf, stem bark and root) of *Nauclealatifolia,* showed variations in their phytochemicals and their antioxidant potential as presented in Figs. 1 to 4.

Fig. 1 presents the result for the quantitative phytochemical analysis of the leaves, bark and root of *N. latifolia*. The result showed the presence of alkaloid, flavonoid, tannin and saponin. The result indicated that the stem bark contained the highest amount of alkaloid (2.58 g/100g), while the root had the lowest amount of alkaloid (0.88 g/100g). Alkaloids have been reported to have analgesic properties (Omoyeni and Adeyeye, 2009).The result further showed that the leaf extract was very rich in flavonoid (0.83 g/100g), while the stem bark had the highest content of tannin (0.53 g/100g) and saponin (0.84 %). Saponins are responsible for the haemolytic properties of plant parts. This confers to the plant the traditional medicinal function as a cholesterol binding agent (Coe and Anderson, 1996; Giovanmucci, 1998). Saponin has a low value 0.56 (leaves), 0.84% (stem bark), and 0.72% (root).The values are very low when compared with the reported values for *Nauclealatifolia* stem bark and root 12.01% and 5.57%, respectively by (Egbung*et al*., 2013).The wide difference observed may be due to seasonal variation, collection time and extraction methods. The value obtained for the leaf can also be compared to the reported value for *Nauclealatifolia* leaves 1.25% (Eze*et al*., 2014). Saponin also has a relationship with sex hormones involved in controlling the onset of labour in women and subsequent release of milk called oxytocin (Okwu and Okwu, 2004). Saponin assists in combating bacterial infections and counter fungus and viruses and have been shown to compliment the effectiveness of some vaccines (Agoha, 1981; Osunwole, 1999). Therefore the leaves of *Nauclealatifolia*may prove to be useful in treating difficult fungal and yeast infections. This supports the use of the plant in treating sexually transmitted diseases such as gonorrhoea, syphilis and herpes in herbal medicine (Okwu and Okwu, 2004; Farquar, 1996).

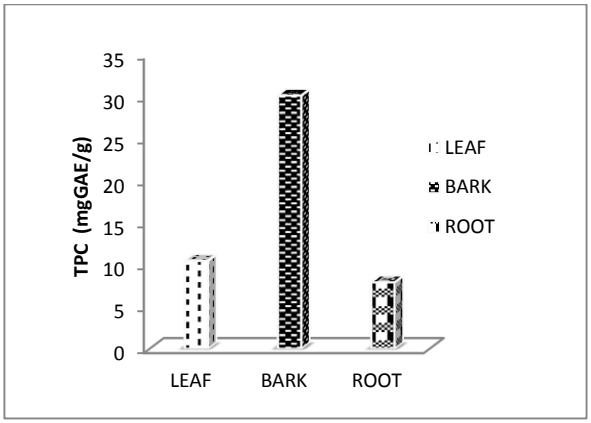

Fig. 1: The total phenolic content of *Nauclealatifolia.*

Flavonoids are biologically active phytochemicals whose functions include anti-inflammatory, antiallergic and anti-tumour agents. Some flavonoids e.g. isoflavones relieve hay fever, eczema, sinusitis and asthma, as well as reduce blood cholesterol and can prevent osteoporosis as well as ease menopause symptoms (Bohm and Kocipai-Abyazan, 1994). Flavonoids are free radical scavengers and are super antioxidant as phenolics which are water soluble and prevent oxidative cell damage and have strong anti-cancer properties (Salah *et al.*, 1995; Paul*et al*, 2012). The flavonoid content of the different parts ranged between 0.08-0.83%. The presence of flavonoid in the leaves, bark and root of *Nauclealatifolia*supports its ethnomedicinal use. Flavonoids also show antimicrobial activities (Cushnie and Lamb, 2009).

Tannins possess astringent properties and hasten the healing of wounds and inflamed mucous membrane (Bohn and Kocipai-Abyazan, 1994). The tannin content of the stem bark (0.53 g/100g) and root (0.13 g/100g) of *Nauclealatifolia*respectively has a low value when compared with result reported by Egbung*et al*., 2013 for the bark (2.12%) and root (2.25%) of *Nauclealatifolia.* The tannin content support the use of the plant for treating wounds, various ulcers, haemorrhoids, frost bite and burn in herbal medicine because of antibacterial effects of tannins (Akiyana*et al*., 2011) as well as treating inflamed throats, mouth and as a veterinary intestinal astringents (Agoha, 1981).

The presence of these phytochemicals could contribute to the ethnomedicinal usage of the plant to treat various ailments.

Phenolic compounds are found usually in both edible and non-edible plants with several biological effects which include antioxidant activity (Ayeleso *et al.*, 2014). In this study, the total polyphenolic contents of the extracts of the leaves, stem bark and root were determined using the diluted Folin-Ciocalteu reagent. The total polyphenolic content was higher in the stem bark than the root and leaves with mean values of 30.16, 7.90 and 10.47 g/100g, respectively. Generally, the result showed that all the tested plant parts are rich in total polyphenols (Fig. 2).

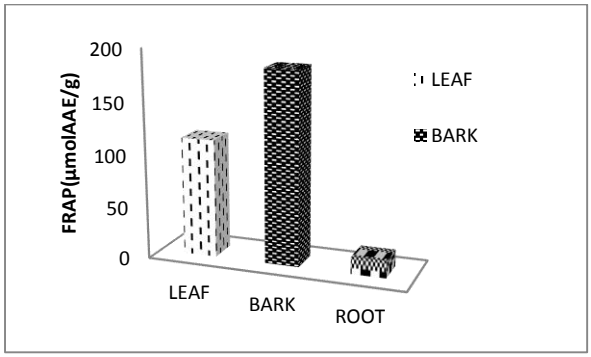

Fig. 2: The FRAP antioxidant activity of*Nauclealatifolia.*

The DPPH Radical Scavenging Activity in the extracts of the leaves, stem bark and root of *Nauclealatifolia*are presented inFig. 3. DPPH radical is used as a stable free radical to determine the antioxidant activity of natural compounds and the scavenging of stable radical (DPPH) is considered a valid and easy assay to evaluate scavenging activity of antioxidants (Suhaj, 2006; Maizura*et al.*, 2011). In this assay, purple colour of DPPH is reduced to α,α-diphenyl-β-picrylhydrazine (yellow coloured) when neutralized. The extent of the change in colour is proportional to the concentration and strength of the antioxidant (Saeed*et al*., 2012).

In this study, the three plant parts displayed antioxidant activity via free radical scavenging activities. The stem bark had the highest activity (30.59 mg/mL) while the root had the lowest activity (3.75 mg/mL) as shown in Fig. 3. The effect of antioxidants on DPPH could be due to their hydrogen donating ability (Gherraf*et al*., 2011).

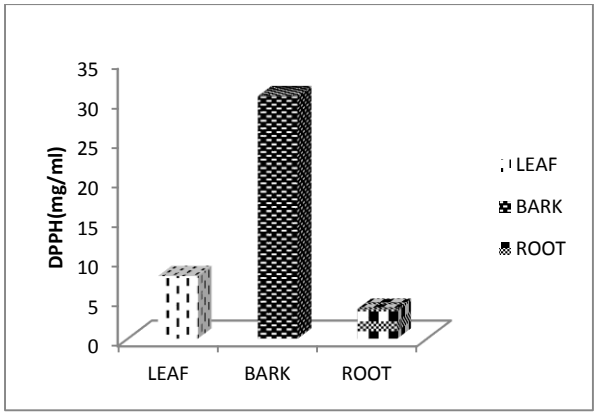

Fig. 3 The DPPH activity of *Nauclealatifolia*

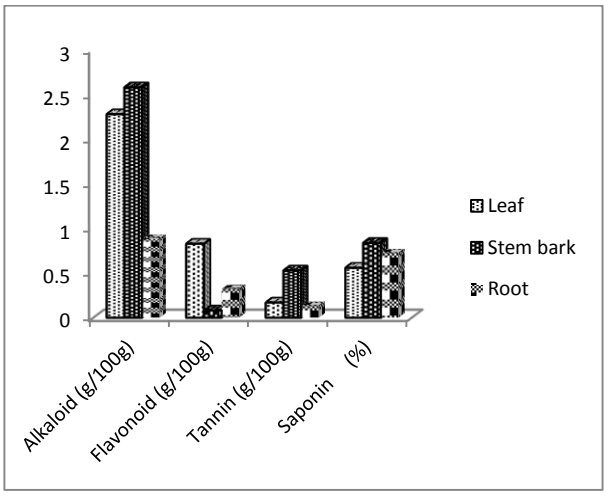

Fig. 4: The phytochemical constituents of *Nauclealatifolia*

The Ferric reducing antioxidant power in the ethanol extracts of the leaves and fruits of *Nauclealatifolia*are presentedinFig. 4. The extent at which the aqueous/ethanolic extract of the leaves and fruits of *Nauclealatifolia* could reduce ferric ions was carried out with FRAP assay. The action of electron donating antioxidants causes a change in the absorbance at 593 nm due to the formation of blue colouredFe^{2+}tripyridyltriazine (TPTZ) compound from the colourless oxidized Fe^{3+}. The stem bark extract showed higher FRAP (184.64 mg/GAE/g sample) than the extract of the leaf and root (114.08 and 16.4184.64 mg/GAE/g sample) respectively as shown in Fig. 4.

Conclusion

Medicinal plants are cheaper than orthodox medicine which are not only very expensive but are also not readily available to the rural farmers (Atata*et al*., 2005). There is therefore a need for interest in the study of medicinal plants and the validation of herbs used in various localities in order to incorporate them into the healthcare system especially since more people are placing emphasis on natural products over synthetic drugs. This policy if pursued will assist to promote the spirit of plant conservation. From the results of the phytochemical analysis, we can conclude that the leaves, stem bark and root of *Nauclealatifolia*are important sources of important phytochemicals and phytonutrients which are in harmony with their use in ethnomedicine. The leaves, stem bark and root of the plant are also of significant antioxidant value and can be exploited in the development of neutraceuticals.

References

Agoha RC 1981. Medical Plants of Nigeria. Offset DrukkerijiFaculted Der Wiskunde en Natuorwetens, Chappens, Nijimegen, Netherland, p 10.

Anwar F, Jamil A, Iqbal S & Sheikh MA 2006. Antioxidant activity of various plant extracts

under ambient and accelerated storage of sunflower oil," *GrasasyAceites*, 57(2): 189 - 197.

Akiyana H, Fuju K, Yamaski O, Ono T &Iwatsuki K 2011. Antibacterial action of several tannins against staphyloccusaureus.*J. Antimicro. Chemotherapy*, 48(4): 487-491.

Ayeleso AO OguntibejuOO& Brooks NL 2014.*In vitro* study on the antioxidant potentials of the leaves and fruits of *Nauclealatifolia.The Scientific World Journal*, Article ID 437081, 8p.

AtataRFSani A &Adewole SM 2005. Effects of stem bark extract of *Enantiachlorantaon* some clinical isolates. *Biokemistri*, 15(2): 84 – 92.

BalunasMJ&Kinghorn AD 2005. Drug discovery from medicinal plants.*Life Sciences*, 78: 431-441.

Basu S 2010. Fatty acid oxidation and isoprostanes: Oxidative strain and oxidative stress. *Prostaglandins, Leukotrienes& Essential Fatty Acids*, 82: 219-225.

Benzie IFF& Strain JJ 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem*., 239: 70–76.

Bernard S, Chang MD, Daniel H, Lowenstien MD. Mechanisms of disease epilepsy.*The New England J. Medicine*, 349: 1257–66.

BöhmBA&Kocipai-Abyazan R 1994.Flavonoid and condensed tannins from leaves of *Vaccinumraticulation*&*Vaccinumcalcyimium*.*Pacific Sciences*, 48: 458-63.

Coe FC & Anderson GJ 1996. Screening of Medicinal plants for bioactive compounds. *J. Food Techn.*, 3(9): 429 – 431.

CushnieTPT& Lamb AJ 2009.Antimicrobial activity of flavonoids.*Int. J. Antimicro. Agent*, 26(5): 343-356.

DevasagayamTPATilakJCBoloorKK Sane KS Ghaskadbi SS &Lele RD 2004. Free radicals and antioxidants in human health: current status and future prospects. *J. Assoc. Physicians of India*, 52: 794–804.

Donalisio M, Nana HM, Ngane RAN, Gatsing D, Tchinda AT, Rovito R, Cagno V, Cagliero C, BoyomFF, Rubiolo P, Bicchi C &Lembo D 2013. In vitro anti-Herpes simplex virus activity of crude extract of the roots of *Nauclealatifolia* Smith (Rubiaceae).*BMC Complem.&Altern. Medicine*, 13: 266.

Egbung GE, AtangwhoIJ, Iwara IA, Odey MO &Ebong PE 2013.Chemical composition of root and stem bark extracts of*Nauclealatifolia*.*Appl. Sci. Res.*, 5(3): 193-196.

Eze SO & Ernest O 2014.Phytochemical and nutrient evaluation of the leaves and fruits of *Nauclealatifolia* (Uvuru-ilu).*Communi.in Appl. Sci.*, 2(1): 8-24.

FaleyeFJ&Akinwunmi OA 2016. Comparative nutritional compositions of the leaves, bark and root of *Nauclealatifolia*. *J. Medical & Bio. Sci. Res.*, 2(7): 127-130.

FarquarJW 1996. Plant sterols: their biological effects in humans. *Handbook of Lipids in Human Nutrition*: BOCA Rotan HrCRC Press, pp. 101-105.

Gherraf N, Ladjel S, Labed B &Hameurlaine S 2011. Evaluation of antioxidant potential of various extracts of *Traganumnudatumdel*. *Plant Sci. Feed*, 1(9): 155-159.

Giovannucci E 1998. Plant Bioactive Components: Phytochemistry. *Bio. Res.*,33: 159–165.

HarborneJB 1973. *Phytochemical Methods*: A Guide to Modern Techniques of Plant Analysis. Chapman A & Hall, London, p. 279.

James O &UgbedeHH 2011.Hypocholesterolemic effects of *Nauclealatifolia*(Smith) fruit studied in albino rats. *Am. J. Tropical Medicine & Public Health*, 1(1): 11–21.

Khalaf NA, Shakya AK, Al-Othman A, El-Agbar Z & Farah H 2008. Antioxidant activity of some common plants.*Turkish J. Bio*., 32(1): 51–55.

Khalid S, RizwanGH, Yasin H, Perveen R, Abrar H, Shareef H, Fatima K & Ahmed M 2013. Medicinal importance of *Holoptelaintegrifolia*(Roxb). Planch - Its biological and pharmacological activities. *Natural Products Chem. & Res.*, 2(1): 1-4.

Paul K, Jourma K, Ritva J, Harri H, Reunaner MA, Timo H &Arpo A 2012. Flavonoid intake and risk of chronic diseases.*Amy Clinical Nutrition*, 76(3): 560 – 568.

Obadoni BO &Ochuko PO 2001.Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria.*Global J. Pure & Appl. Sci.*, 8: 203-208.

OjongLJ, Abdou JP, Kandeda AK, YayaAJG , Tchamgoue AD, TchokouahaLRY, Nkantchoua NGC, Agbor GA, Moyou RS & Ngo EB 2016. Anticonvulsant and in vitro antioxidant activities of *MomordicaCissoides*L. (Cucurbitaceae).*J. Appl. Pharmac. Sci.*, 4: 117-123.

Okwu DE &Okwu ME 2004.Chemical composition of Spondiasmombinlinn plant parts.*J. Sustainable Agric. &Envt*.,6(2): 140-147.

Omoyeni OA &AdeyeyeEI 2009.Chemical composition, calcium, zinc and phytate interrelationships in Aervalanata (Linn) Juss.exSchult leaves. *Oriental J. Chem*.,25(3): 485.

Osunwole SA 1999.Traditional medicinal uses of selected plants on the University of Ibadan campus.*The Nigr. Field*, 64:168 – 173.

Maizura M, Aminah A & Wan Aida WM 2011. Total phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (*Zingiberofficinale*) and turmeric (Curcuma longa) extract. *Int. Food Res. J.*, 18(2): 529-34.

Nkafamiya II, ManjiAJ, ModibboUU&Umaru HA 2006.Biochemical evaluation of *Cassipoureacongoensis*(Tunti) and *Nuclealatifolia*(Luzzi) fruits.*Afr. J. Biotech.*, 5(24): 2461–2463.

NyaaTBL, Tapondjou AL, Barboni L, TamokouJDD, Kuiate JR, Tane P & Park HJ 2009. NMR assignment and Antimicrobial/antioxidant activities of 1β-hydroxyeuscaphic acid from the seeds of Butyrospermumparkii.*NaturalProductSci.*, 15(2): 76À82.

Pearson D 1976. *The Chemical Analysis of Foods*.3rd edition.

Saeed N Khan MR&Shabbir M 2012. "Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilisleptophylla*L. *BMC Complem. &Altern. Medicine*, 12: 221.

Schrader M &Fahimi HD 2006.Peroxisomes and oxidative stress.*BiochimBiophysActa*, 1763: 1755-1766.

Škrovánková S, Mišurcová L & Machu L 2012. Antioxidant activity and protecting health effects of common medicinal plants," *Advances in Food and Nutrition Research*, 67: 75– 139.

Suhaj M 2006. Spice antioxidants isolation and their antiradical activity: A review. *J. Food Composition & Anal.*, 19(6): 531-537.

TaïweGS, Bum EN, Talla E, Dimo T, Weiss N, Sidiki N, Dawe A, Okomolo Moto FC, DzeufietPD&Waard MD 2011. Antipyretic and antinociceptive effects of Nauclealatifolia root decoction and possible mechanisms of action. *Pharmaceutical Biology* 49(1): 15-25.

Vinson JA Jang J Dabbagh YA Serry MM &Cai S 1995. Plant phenols exhibit lipoprotein-bound antioxidant activity using an in vitro model for heart disease. *J. Agric & Food Chem.*, 43: 2798–2799.