Research Journal of Chemical Sciences _____ E-ISSN 2231-606X
Vol. **6(9),** 19-26, September **(2016)** *Res. J. Chem. Sci.*

Phytochemical Analysis and Antioxidant Properties of *Lasianthera africana* Leaves, Stems and Roots Extracts

Atiko R.[1*], Onocha P.A.[2] and Oyedemi S.O.[3]
[1]Department of Chemistry, Faculty of Science, Gombe State University, Gombe, Nigeria
[2]Department of Chemistry, Faculty of Science, University of Ibadan, Ibadan, Nigeria
[3]Phytomedicine Research Centre, Botany Department, University of Fort Hare, Alice, South Africa
r_atiko@yahoo.co.uk

Available online at: www.isca.in, www.isca.me
Received 28th June 2016, revised 16th August 2016, accepted 5th September 2016

Abstract

The study evaluated the phenolic content and antioxidant capacities of Lasianthera africana leave stem and root extractswhich is utilized for the management of oxidative stress related ailments in eastern Nigeria. The plant's antioxidant and free radical scavenging properties was evaluated in vitro against ferric reducing agent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide (NO), hydrogen peroxide (H_2O_2) and 2,2-azinobis (3-ethylbenzothiazolie-6-sulphonic acid) diammonium salt (ABTS). The total phenolic content and the total flavonoids of the extracts were determined and their related effect on the antioxidant activity was evaluated. The reducing capacity of the standard drug, butylated hydroxytoluene (BHT) was found to be lower than those of the extracts. The free radical quenching effect of the extracts against DPPH, NO, H_2O_2 and ABTS radicals were concentration dependent with IC_{50} of 0.30 ,0.29, 0.32 and 0.30 mg/mL for the leaves; 0.31,0.29,0.31 and 0.31 mg/mL for the stem; and 0.30, 0.27, 0.31 & 0.30 mg/mL for the roots respectively. The total phenolic content of the extracts were 18.21±0.1, 16.67±0.5 and 19.5±0.3mg GAE/g DW while the total flavonoids were 6.39±0.2, 6.83±0.1, 5.15±0.2mg QE/g DW for the leaves, stem and root extract respectively. Our results showed that Lasianthera africana ethanol extracts are good sources of free radical scavengers and therefore could be used in the management of oxidative disorders.

Keywords: *Lasianthera*, *Africana,* Plant extracts, Antioxidant.

Introduction

The biological system undergoes metabolic processes involving redox reaction which generates reactive oxygen species (ROS) like superoxide radical ($O_2^{\cdot}$), peroxynitrite anion ($ONOO^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\cdot}$) and hydroxyl radical ($OH^{\cdot}$). Under normal circumstances, the ROS generated during metabolic process are detoxified by the antioxidant enzymes in the biological system in order to achieve equilibrium between the ROS generation and its detoxification by antioxidants within the system. But when the generation of ROS becomes greater than the antioxidant defense of the cell, a phenomenon that leads to potential damage occurs. Thus the diminished antioxidants and/or increase in production of ROS results in oxidative destruction of DNA, enzymes, proteins and lipids[1,2] which is the primary cause of many diseases like arthritis, cancinoma, inflammations, asthma, cardiac arrest, diabetes mellitus, fibroids and cognitive disorders[3,4]. These ailments can be slowed down by suppressing the starting or progression of the oxidative chain reaction by the intake of natural or synthetic antioxidants. However, investigations revealed that the synthetic antioxidant drugs in use, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) harmful to mans[5-7]. Plants produce a various free radical quenching compounds like polyphenols, nitrogenous compounds, terpenoids and saponins which scavenge the reactive oxygen species and thus help treat and prevent the diseases associated with ROS[8,9].

Lasianthera africana (P. Beauv) is a glabrous shrub up to four meters high with terete branchlets and white flowers in umbellate head like clusters, found as under storey in secondary jungle and thickets in the rain forest of southern Nigeria, Western Cameroon extending to Zaire[10]. The plant is used for treating diarrhoea, dysentery, stomach troubles, fibroids, parasitic infections, ulcers and diabetes. Its fruits are also used for treatment of asthma, hypertension, skin diseases and wounds healing[11-13]. *L. africana* leaves are reported to posses antiulcerogenic [14], antimalarial[15] and antimicrobial[16,17]. The total phenolics and flavonoids content of the leaves were also reported [18].

There is dart of information on the antioxidant properties of the stem and root extracts of the plant despite its traditional use in the management of oxidative stress diseases. The antioxidant activity of *L. africana* leaves by ferric thiocyanide ethod has also been reported[19] but there no information on the ethanol extract of the plant. We now therefore report the total phenolics and the oxidation inhibition capacities of the ethanol extracts of leaves stem and root of *Lasianthera africana* using various *in vitro* antioxidant methods.

Materials and Methods

Collection of Plant Material: *L. africana* roots, stems and leaves were gathered from a farmland in Cross River State, Nigeria by Mr. Ubong and authenticated by Mr. Wale of Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria. The plant sample was deposited at the Institute with herbarium number FHI 108317.

Preparation of Plant Extracts: The leaves, stems and roots (100 g each) of *L. africana* were collected and dried at ambient temperature then ground using Hammer mill. The samples were separately extracted three times with 95% ethanol 48 hours. The plant extracts were filtered then concentrated at 35°C under reduced pressure by means of rotary evaporator. The crude ethanolic extracts were then used for phytochemical analysis and the antioxidant assays.

Determination of Total Phenolics: Total polyphenols of *L. africana* extracts were estimated by the use of the laid down procedures adopted by Mbaebie *et al* [20]. To 2 mL solution of 75% sodium trioxocarbonate (IV) was added 10% Folin-Ciocalteu reagent (2 mL) followed by 0.5 mL measured volume of the extract at 1mg/mL concentration. This combination was vortexed for 15s followed by incubation for half an hour at 40°C temperature for development of colour. Hewlett Packard UV Spectrophotometer was used to record the absorbance at 765 nm. Using the expression from the calibration curve, the total polyphenolic content was evaluated as mg/g gallic acid equivalent (GAE)/DW (sample dry weight):

$A = 0.1216\ Z,\ R^2 = 0.936512$

Where: Z represents the absorbance readings and A the gallic acid equivalent in mg/g dry wet of the plant sample.

Determination of Total Flavonoids: Ordonez *et al*[21] procedure for evaluating the total flavonoid contents was adopted to estimate the various concentrations of the extracts. The test depends on the complex formation of aluminium with flavonoid molecule. Equal volumes of 2% $AlCl_3$ (0.5 mL) in ethanol and the extract (1mg/mL) were mixed and kept at an indoor temperature for an hour for the development of yellow colouration. The UV-VIS Spectrophotometer was used for regording absorbance at 420 nm. All the readings were recorded in triplicates and the total flavonoid content was estimated as quercetin equivalent (mg/g) using the following equation obtained from the calibration curve:

$A = 0.255\ Z,\ R^2 = 0.9812$

Where: Z represents the absorbance and A the quercetin equivalent.

Determination of Reducing Power: The reducing capacity of an extract serves as significant indicator of its potential antioxidant capacity. The evaluation of the reducing power was carried out according to the method of Kumar and Hemalatha[22], with some modifications. A 1.0 mL of the extract (1.0 mg in 50µL DMSO made up to 1.0 ml with distilled water) was mixed thoroughly with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide (2.5 mL). The same procedure was carried out for BHT and Vitamin C which were used as standard drugs. These mixtures were incubated individually at 50°C for 20 min, followed by addition of trichloroacetic acid (10% w/v) then centrifuged at 3000 rpm for 10 min. The supernatant was collected and mixed with 0.5 mL of 0.1% Ferric chloride solution. Absorbance was measured at 700 nm against a blank solution without the extract.

Determination of NO scavenging capacity: The procedure mapped out by Ebrahimzadeh *et al* [6] was adopted to ascertain the NO scavenging capacity of *L. africana* leave, stem and root extracts. A 2 mL solution of 10 mM $Na_2[Fe(CN)_5NO]$ was made in 0.5 mM phosphate buffer saline (pH 7.4) and added to 0.5 mL different concentration of the extracts (0.1-0.5 mg/mL in methanol), BHT and rutin. The mixtures, individually, were incubated at 25°C for 150 min. A 0.5 mL quantity of each incubated solution was added to 0.5 mL of Griess reagent (solution of 2% H_3PO_4, 1% sulphanilamide and 0.1% Napthylethylenediamine dihydrochloride). The mixture was then incubated at 25°C for 30 min. Absorbance reading was taken at 540 nm and the percentage of NO inhibition capacity of extract was evaluated by the equation:

$$\%\ Inhibition = \left(\frac{[ABS_c - ABS_s]}{ABS_c}\right) \times 100$$

Where ABS_c was the absorbance of NO radical + methanol; ABS_s was the absorbance of NO radical + sample or standard of test.

Determination of DPPH scavenging activity: The method of Shen *et a.,l* [23] was adopted for the estimation of the scavenging capacity of 2,2-diphenyl-1-picrylhydrazyl free radical by *L. africana* extracts. A 1.0 mL solution of 2, 2-diphenyl-1-picrylhydrazyl at 0.135 mM in methanol was added to 1.0 mL of the various concentrations of the extract or the standard rutin separately. The mixtures were vortexed thoroughly and were maintained at indoor temperature in the absence of light, for half an hour after which the absorbances were taken at $\lambda = 517$ nm. The capacity of the plant extracts to abstract the DPPH free radical was mathematically estimated by the following equation:

$$\%\ Inhibition = \left(\frac{[ABS_c - ABS_s]}{ABS_c}\right) \times 100$$

Where ABS_c represents the absorbance of DPPH radical in methanol; ABS_s represented the absorbance of DPPH radical with the sample or standard.

Determination of ABTS scavenging capacity: The procedure mapped out by Re *et al* [24] was adopted to determine the scavenging capacities of the plant extracts against $ABTS^{\cdot+}$

radical cation. Two stock solutions; 7 mM 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), (ABTS) and 2.4 mM potassium persulphate ($K_2S_2O_8$), were prepared and mixed in equal proportions. The mixture was kept in the dark cupboard for 12 hours at ambient temperature. After this, $ABTS^{\cdot+}$ solution was further diluted with deionized water until absorbance reading of 0.706 ± 0.001 units at 734 nm was reached. The various concentration of extracts were added to dilute solutions of the generated $ABTS^{\cdot+}$ radical separately and absorbance was taken after 7 mins. The amount of $ABTS^{\cdot+}$ cation inhibited by the extracts as compared to the reference standard rutin is represented by the equation:

$$\%\ ABTS^{\cdot+}\ Scavenging\ Capacity = \left(\frac{[ABS_c - ABS_s]}{ABS_c}\right) \times 100$$

Where ABS_c was the absorbance of $ABTS^{\cdot+}$ radical + methanol; ABS_s was the absorbance of $ABTS^{\cdot+}$ radical + sample or standard.

Hydrogen peroxide scavenging activity: The method of Ruch *et al* [25] was used to determine the capacity of *L. africana* extracts to scavenge hydrogen peroxide. The plant extracts prepared at different concentrations (0.1 – 0.5 mg/mL) in 4 mL methanol were mixed separately with 0.6 mL of 40 mM Hydrogen peroxide which was made in a phosphate buffer (0.1 M) at pH 7.4. After this, the mixture was developed for 10 min. The reading of absorbance was carried out at 230 nm against a blank Phosphate buffer. This same procedure was repeated for the standards: Rutin and BHT. The amount of hydrogen peroxide scavenged by *L. africana* extracts was estimated by the expression:

$$\%\ [H_2O_2]\ Scavenged = \left(\frac{[ABS_c - ABS_s]}{ABS_c}\right) \times 100$$

Where ABS_c was the absorbance of H_2O_2 + methanol; ABS_s was the absorbance of H_2O_2 + sample or standard.

Results and Discussion

Total Phenolics: Phenolic compounds (tannins, flavonoids and proanthocyanidins) are group of natural products synthesized by plants as they adapt themselves to biotic and abiotic stress circumstances such as, drought, high ultra-violet light, infection and winter[26]. These compounds have been reported to have vasoconstriction effect on small superficial vessels; antimicrobials, antioxidants, anti-inflammatory, antiviral, anti-diarrhoeal, antimutagenic and chemopreventive[27]. Phytophenolics are reported to be involved in tissue regeneration in wounds and burns by preventing loss of fluid[28]. The findings of our investigation revealed high concentration of total phenolic content of all the *L. africana* ethanol extracts tested, ranging from 16.6 to 19.5 mg of GAE/g dry weight of extract (Figure-1). The root extracts had the highest concentration (19.5±0.3 mg of GAE/g DW) while the stem exhibited the lowest concentration (16.66±0.2 mg/mL). The total flavonoids content of the leaves, stems and roots of the plant extracts were 6.39± 0.2, 6.83±0.1 and 5.15±0.2 mg of QE/g DW respectively. Our results agree with the previous studies which reported that the total phenolic content of the plant's root extract was higher than that of the stem bark and leaves[29]. However, our results for total phenolic content and total flavonoids are significantly higher than those obtained in literatures for *L. africana* leaves[18]. The high phenolic content exhibited by this plant extracts may be the primary cause for the strong antioxidant properties detected in this study.

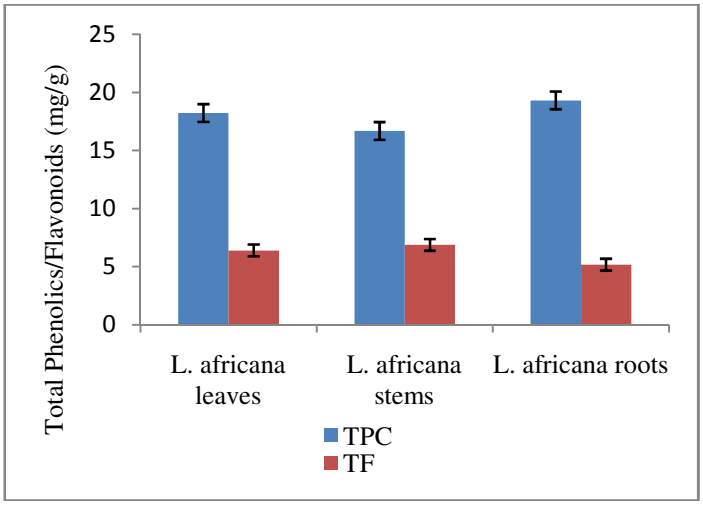

Figure-1
Total polyphenolic (TPC) and flavonoids (TF) content of the ethanol extracts of *L. africana* leaves, stems and roots. The results are means ± SD (n=3)

Reducing Power: The antioxidant effects of ethanol extracts of *L. africana* leaves, stems and roots were determined through a series of chemical parameters in comparison to known antioxidants (rutin, BHT and ascorbic acid). The antioxidant properties of plant extracts cannot be ascertained by only one procedure because of the complex nature of secondary metabolites present; therefore, the commonly accepted assays involving in vitro conditions were employed in this study. The antioxidant activities of *L. africana* extracts were evaluated by measuring its capacity to reduce Fe^{3+} to Fe^{2+}. The result showed that the reducing power of *L. africana* leaves, stem and roots extracts and the standard drugs [Vitamin C and butylated hydroxyl toluene (BHT)] displayed an increase in activity as concentration increased (Figure 2). The various concentrations of ethanol extracts of the plant showed a reducing power comparable to the standard drug ascorbic acid and relatively higher than BHT. *L. africana* roots exhibited the highest reducing power with absorbance of 0.57 nm at 0.3 mg/mL while the stems exhibited the lowest of 0.37 nm at the same concentration. This result suggest that the polyphenolic compounds of *L. africana* play an important role in reducing ferric ions to ferrous ions with the root extract exhibiting the highest reducing power IC_{50} of 0.37 mg/mL. Similar trend has been reported for some sea plants[30].

DPPH Quenching Capacity: DPPH is a stable free radical because it delocalizes a spare electron over the molecule as a whole. This delocalization of electron gives it deep violet colour that absorbs at 517nm. DPPH in a solution can accept hydrogen atom or electron from an antioxidant; it gets itself reduced to yellow colour. Figure-3 depicts the results of DPPH radical quenching activities of *L. africana* ethanol extracts as compared to that of the reference standard; rutin and BHT. The plant extracts exhibited a significant dose dependent quenching of DPPH radical so much so that at 0.5 mg/mL, the quenching activities of the leaves stem and roots were 85%, 84%, 89% respectively as compared to standard, rutin 95%. The strong inhibition activities displayed by the plant extracts on DPPH radical could be related to high phenolic content of the extracts which are capable of donating electrons that scavenge the free radicals. Thus, *L. africana* ethanol extracts could be promising source of therapeutic agents for the management of stress induced conditions.

NO Scavenging Activity: Nitric oxide (NO) is a main molecule that sends signal to the body cells in order to curtail pathogenesis of various diseases associated with inflammations such as diabetes mellitus, carcinomas, ulcers and arthritis[20,31]. NO is generated *in situ* from sodium nitroprusside ($Na_2[Fe(CN)_5NO]$) at pH 7.4 and this reacts with oxygen to form a cell damaging nitrite[20]. The scavenging activity of *L. africana* extracts against NO was evaluated and the results are presented in Figure-4. The percentage at which the plant extracts (leaves, stem and root) and rutin inhibited the nitric oxide radical at 0.3 mg/mL were 52%, 47%,51and 59% while their IC_{50} (concentration of antioxidant needed to scavenge 50% of free radicals) values were 0.29, 0.29, 0.27 and 0.28 mg/mL respectively (Table-1). All the plant extracts exhibited a good NO radical scavenging activity which was in comparison to the reference standard rutin; with *L. africana* stems exhibiting the highest activity. This observation gives an indication that the ethanol extracts of *L. africana* leaves, stems and roots have strong antioxidant potential and therefore supports its folkloric use for the treatment of inflammations and wounds.

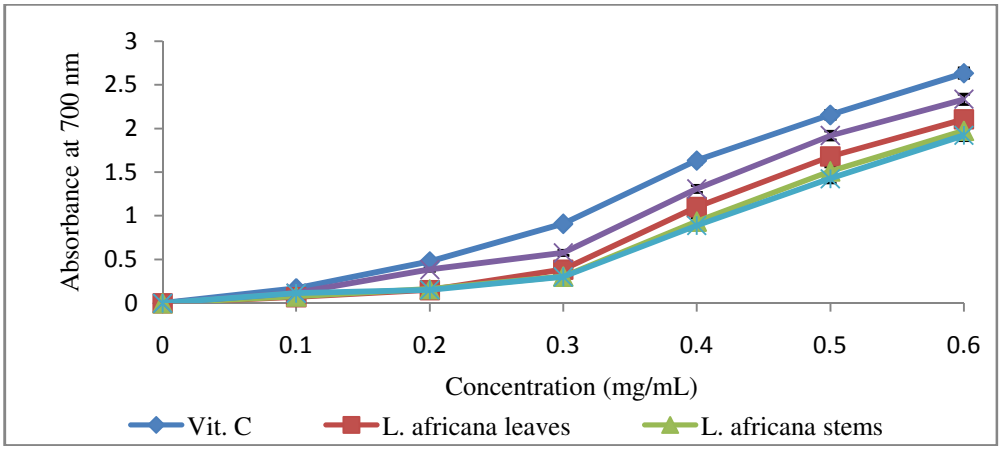

Figure-2
Reducing power activities of leaves stems and roots ethanol extracts of *L. africana* in comparison to Ascorbic acid (Vit. C) and BHT

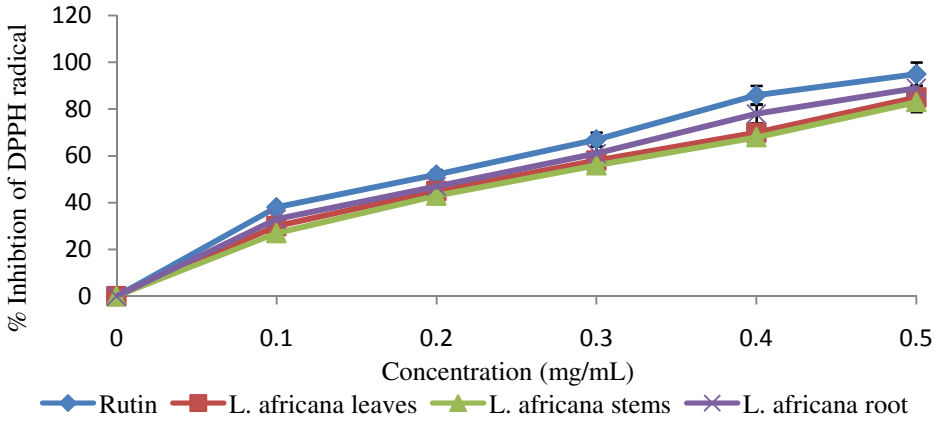

Figure-3
DPPH radical scavenging activity of ethanol extracts of *L. africana* leaves, stems and roots

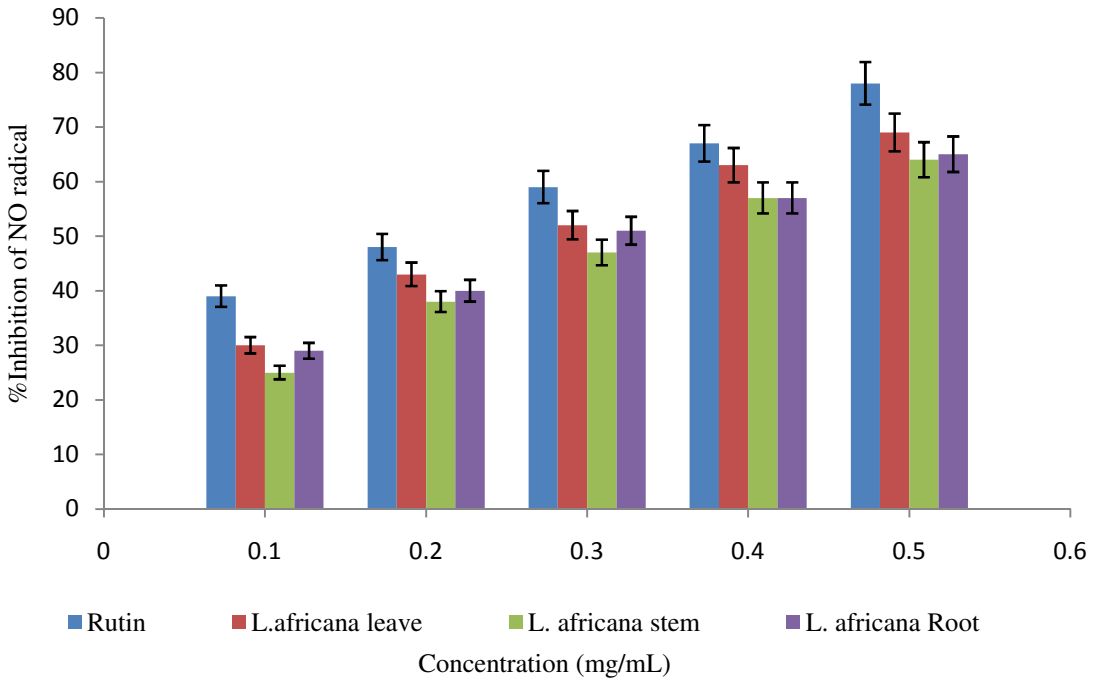

Figure-4
Nitric Oxide scavenging activities of the ethanol extracts of *L. africana* leaves stem and root in comparison to rutin

Table-1
Scavenging activities of *L. africana* leaves stem and roots ethanol extracts

SAMPLE	DPPH		NO		H_2O_2		ABTS	
	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2	IC_{50}	R^2
Rutin	0.26	95.4	0.28	89.2	0.29	73.9	0.29	89.4
L. africana leaves	0.30	96.9	0.29	91.5	0.32	80.1	0.30	93
L. africana stems	0.31	96.6	0.29	92.3	0.31	81.5	0.31	92.4
L. africana roots	0.30	97.7	0.27	94.5	0.31	78.8	0.30	92.6
BHT	n/d	n/d	n/d	n/d	0.33	80.1	n/d	n/d
Vit. C	n/d	n/d	n/d	n/d	n/d	n/d	n/d	n/d

n/d:- not determined; Vit. C: - Ascorbic acid, IC_{50}:-concentration (mg/mL) of antioxidant that exhibits 50% quenching capacity.

Hydrogen Peroxide Quenching Capacity*:* Hydrogen peroxide is of great importance when we consider ROS because of its ability to permeate cell membranes and thus slowly oxidize some compounds. H_2O_2 on its own is unreactive but when it is converted to hydroxyl radicals in the presence of metal ions, it becomes the most devastating ROS[32]. Figure-5 displayed the percentage inhibition of hydrogen peroxide by the ethanol of extracts *L. africana* leaves, stem and roots. The decomposition of $H_2O_2$by the plant's ethanol extracts were observed in a concentration dependent manner. At 0.3 mg/mL, the percentage decomposition of H_2O_2 by the plant leaves, stem and roots were 72, 73 and 74 respectively which compares well to the standard BHT (72) but significantly lower than that of rutin (89). This suggests that *L. africana* extracts may be containing phenolic compounds that structurally differ from rutin or BHT which donates hydrogen to H_2O_2 and thereby rendering it ineffective by forming water [33]. The scavenging effect of different extracts on H_2O_2 increased in the following order: leaves > stems > roots, implying that the stems of *L. africana* could be a better candidate for management of ROS induced diseases.

ABTS Scavenging Activity: $ABTS^{\cdot+}$ radical was formed by reaction of ABTS with potassium persulphate within 12 hours of incubation. This reaction formed a blue chromophore that was decolorized by the various ethanol extracts of *L. Africana.* The free radical quenching abilities of the extracts increased as their concentration increases. Figure-6 showed the scavenging activities of *L. africana* extracts against $ABTS^{\cdot+}$ radical. The $ABTS^{\cdot+}$ radical quenching capacities observed for the extracts were similar to that of the standard drug rutin. the leaves, stems and roots extracts demonstrated IC_{50} magnitude of 0.30, 0.31 and 0.30 mg/mL respectively; at the same time, the IC_{50} of the standard rutin was 0.29 mg/mL, Table 1. The root extract demonstrated the highest $ABTS^{\cdot+}$ radical scavenging capacity, whereas the stem extract exhibited the least acivity. The scavenging potential of the three extracts of *L. africana* on $ABTS^{+}$ radical at high concentration (0.5 mg/mL) were similar to that of DPPH radical. This observation is contrary to what other researchers have reported that plants with DPPH scavenging capacity may not be able to abstract $ABTS^{\cdot+}$ radical[20]. This difference in observation could be due to their solubility in different solvent system and the nature of substrate used [30, 20]. These results correspond with the reports obtained by Wang *et al* and Devi *et al* for *Salvia officinalis* [30, 34].

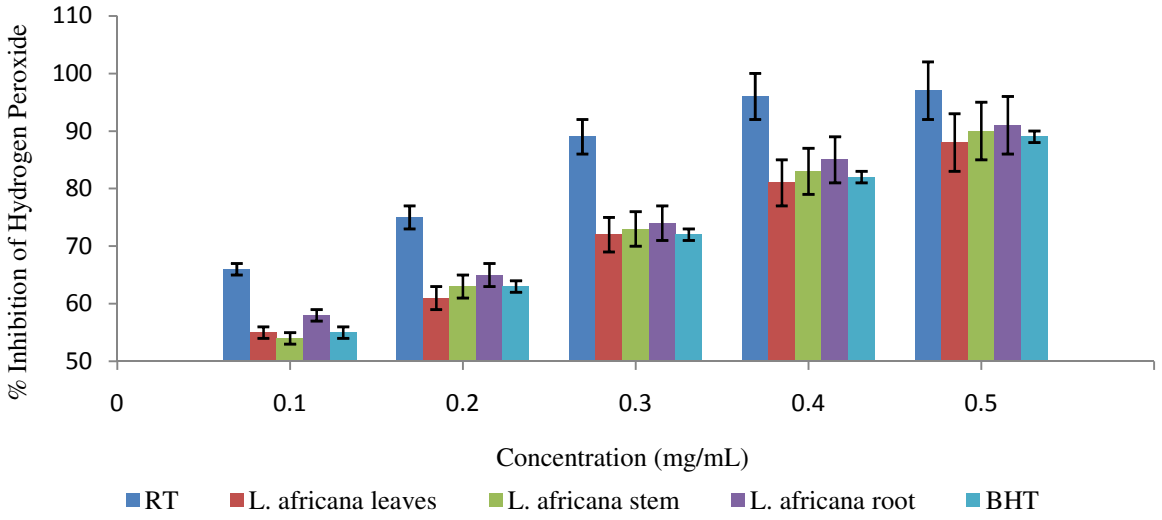

Figure-5
Hydrogen peroxide scavenging activities of ethanolic extracts of *L. africana* leaves, stems and roots in comparison to BHT and rutin (RT)

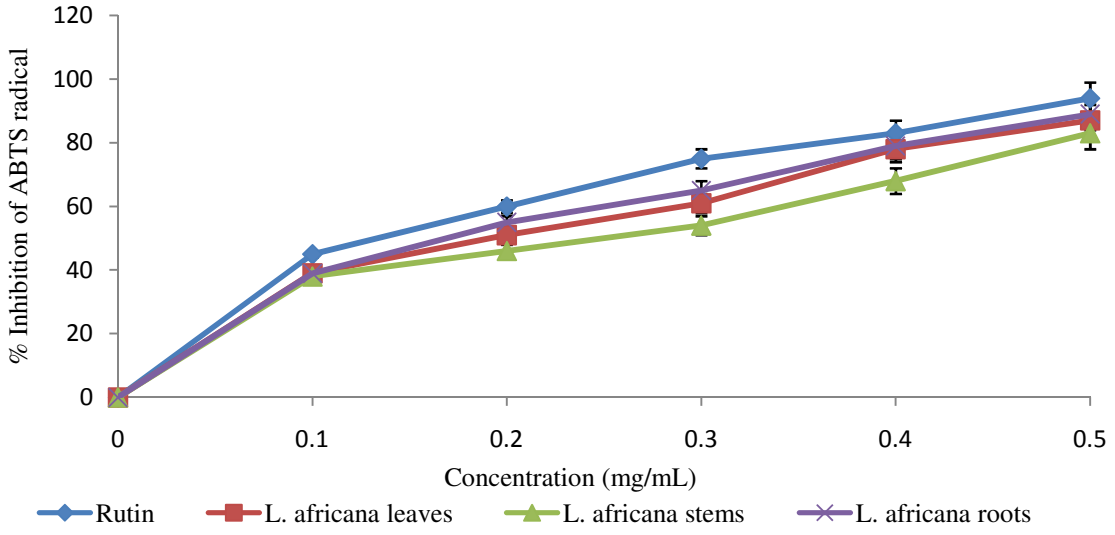

Figure-6
$ABTS^{\cdot+}$ scavenging capacities of ethanolic extracts of *L. africana* leaves, stems and roots in comparison to the standard, rutin

Conclusion

The remarkable antioxidant activities displayed by *L. africana* leaves, stems and root extracts provided justification for the use of the plant by Nigerian traditional healers for the treatment of ailments like ulcers, diabetes, wound binding and inflammations. The root extract displayed the highest total phenolic content and has the highest antioxidant activity against DPPH, ABTS and NO radicals and therefore it stands a better source of natural antioxidant s. Further work is being carried out to isolate the active compounds responsible for these activities as well as to evaluate their toxicity levels.

Acknowledgements

This work was financially assisted by Educational Trust Fund (ETF) through Gombe State University, Nigeria.

Reference

1. Halliwell B. and Gutteridge J.M.C. (2007). Free radicals in Biology and Medicine. Oxford University Press, Oxford, 440-61.

2. Martin F.L., Williamson S.J.M., Paleologon K.E., Hewitt R., El – Agnaf O.M.A. and Allsop D. (2003). Fe(II) induced DNA damage in α –synucleintransfected human dopaminergic BE(2)-MI7 neuroblastoma cells: Detection by the Comet assay. *J. Neurochem*., 87, 620-636.

3. Raghuveer C. and Tandon R.V. (2009). Consumption of Functional food and health concerns. *Pak J Physiol,* 5(1).

4. Ng F., Berck M., Dean O. and Bush A.L. (2008). Oxydative stress in psychiatric disorder: Evidence base and therapeutic implications. *Int. J. Neuropsychopharmacol*., 11(6), 851-876.

5. Lobo V., Patil A., Phatak A. and Chandra N. (2010). Free radicals antioxidants and Functional foods: Impact on human health. *Pharmacognosy reviews*, 4(8), 118-126.

6. Ebrahimzadeh M.A., Pourmorad F. and Hafezi S. (2008). Antioxidant activities of Iranian corn silk. *Turk. J. Biol*., 32, 43-49.

7. Patel V.R., Patel P.R. and Kajals K. (2010). Antioxidant activity of some selected medicinal plants in western region of India. *Advances in Biological Research.,* 4(1), 23-26.

8. Oyedemi S.O., Bradley G. and Afolayan A.J. (2011). In vitro and in vivo antioxidant activities of aqueous extract of Strychnos henningsii Gilg. *Afri. J. Pharm. Pharmacol*., 4(2), 70-78.

9. Ponou B.K., Teponno R.B., Ricciutelli M., Quassinti L., Bramucci M., Lupidi G., Barboni L. and Tapondjou L.A. (2010). Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A.chev. *Phytochemistry,* 71, 2108-2115.

10. Burkill H.M. (1985). The Useful Plants of West Tropical Africa. Edition 2, 1, Families A-D. Royal Botanic Gardens, Kew., 960.

11. Jiofack T., Ayissi I., Fokunang C., Guedje N. and Kemcuze V. (2009). Ethnobotany and phytomedicine of the upper Nyong Valley Forest in Cameroon. *Afri. J. Pharm. Pharmacol.,* 3(4), 144-150.

12. Isong E.U. and Idiong U.I. (1997). Comparative studies on the nutritional and toxic composition of three varieties of Lasianthera africana. *Plant Foods Hum. Nutr*., 51, 79-84.

13. Ajibesin K.K., Ekpo B.J., Bala D.N., Essien E.E. and Adesanya S.A. (2008). Ethnobotanical survey of Akwa Ibom State of Nigeria. *J. Ethnopharmacol.,* 115(3), 387-408.

14. Okokon J.E., Antia B.S. and Umoh E.E. (2009). Antiulcerogenic activity of ethanolic leaf extract of Lasianthera africana. *Afr. J. Tradit. Complement. Altern. Med*., 6(2), 150-154.

15. Okokon J.E., Antia B.S., Essiet G.A. and Nwidu L.L. (2007). Evaluation of in *vivo* antiplasmodial activity of ethanolic extract nof *Lasianthera africana*. *Research J. Pharmacol*., 1(2), 30-33.

16. Andy I.E., Eja M.E. and Mboro C.I. (2008). An evaluation of the antimicrobial potency of *Lasianthera Africana* (BEAUV) and *Heinsia crinata* (G.Taylor) on *Escherichia coli*, *Salmonella typhi Staphylococcus aureus* and *Candida albicans*. *Malaysian J. Microbiol*., 4(1), 25-29.

Pakistan J. Physiol. 5(1): 76 – 83.

17. Ita A.Y. (1996). Screening of plants parts for fungicidal properties. *Trans. Nig. Soc. Bio. Conserv*., 4, 26-40.

18. Ita B.N. (2010). Evaluation of the role of solvents on the extractable content of total phenolics and flavonoids in Nigerian fruits /vegetables. *Nig. J. Agric. Food Environ*. 6(1&2), 29-32.

19. Odukoya O.A., Inya-Agha S.I., Segun F.I., Sofidiva M.O. and Ilori O.O. (2007). Antioxidant activity of selected Nigerian green leafy vegetables. *Am. J. Food Technol*., 2(3), 160-175.

foods: Impact on human health. *Pha*

20. Mbaebie B.O., Edeoga H.O. and Afolayan A.J. (2012). Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. *Asian Pacific Journal of Tropical Biomedicine*., 1, 118-124.

21. Ordonez A.A.L., Gomez J.D., Vattuone M.A. and Isla M.I. (2006). Antioxidant activities of *Sechium edule* (Jacq). *Food Chem*., 97, 452-458.

22. Kumar R.S. and Hemalatha S. (2011). In vitro antioxidant activity of alcoholic leaf extract and subfractions of *Alangium lamarckii* Thwaites. *J. Chem. Pharm Res.,* 3(1), 259-267.

23. Shen Q., Zhang B., Xu R., Wang Y., Ding X. and Li P. (2010). Antioxidant activities of Selenium-contained

protein from the Se-enriched *Biofidobacterium animalis* 01. *Anaerobe,* 16(4), 380-386.

24. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radic. Biol. Med*., 26, 1231-1237.

25. Ruch R.J., Cheng S.J. and Klaunig J.E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis,* 10, 1003-1008.

26. Oboh G. and Rocha J.B.T. (2007). Polyphenols in red pepper [*Capsicum annuum var. aviculare* (Tepin)] and their protective effect on some Pro-oxidants induced lipid peroxidation in brain and liver. *Eur. Food Res. Technol.,* 225(2), 239-247.

27. Makkar H.P.S., Sidhuraju P. and Becker K. (2002). Plants secondary metabolites. Humana Press Inc., New Jersey, 248-253.

28. Okuda T. (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. *Phytochemistry,* 66(17), 2012-2031.

29. Dhalwal K., Deshpande Y.S. and Purohit A.P. (2007). Evaluation of *In Vitro* antioxidant activity of *Sida rhombifolia* (L.) ssp. *retusa* (L). *J. Med. Food*, 10(4), 683-688.

30. Devi G.K., Manivanna K., Thirumaran G., Rajathi F.A.A. and Anantharama P. (2011). In vitro antioxidant activities of selected seaweed for southeast coast of India. *Asian Pacific J. Trop. Med.*, 205-211.

31. Gates P.E. and Strain W.D. et. al. (2008). Human endothelial function and microvascular aging. *Exp. Physiol*., 94, 311-316.

32. Gülcin I. (2006). Antioxidant and antiradical activities of L-carnitine. *Life Sciences*, 78(8), 803-811.

33. Mathew S. and Abraham T.E. (2006). In vitro antioxidant activities and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. *J. Food Chem. Toxicol*., 44, 198-206.

34. Wang M., Li J., Rangarajan M., Shao Y., La Voie E.J., Huang T. and Ho. C. (1998). Antioxidative phenolic compounds from sage (Salvia officinalis). *J. Agric. Food Chem*., 46, 4869-4873.