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EVALUATION OF ANTIOXIDANT POTENTIALS IN METHANOL EXTRACT OF GONGRONEMALATIFOLIUM AND LASIATHERAAFRICANA LEAF

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ABSTRACT

Antioxidants are substances which are capable of neutralizing free radicals and their actions. Oxidative stress is a disturbance in the balance between the production of the reactive oxygen species or free radicals and antioxidant defense which may lead to tissue injury. The aim of this work was to evaluate the antioxidant potentials of two widely used medicinal plants; *Gongronema latifolium* and *Lasianthera africana*, using the DPPH radical scavenging spectroscopic method. From the result obtained *Gongronema latifolium* had a mean scavenging activity of 80.95±0.09 which was not significantly different ($p>0.05$) from that of Vitamin C tablet (93.93±0.12). Similarly there was no significant difference ($p>0.05$) between the mean free radical scavenging activity of *Lasianthera africana* which was 77.32±0.13 and that of vitamin C tablet. Therefore the two plants have appreciable antioxidant property that should not be neglected and their usage encouraged to help mitigate deleterious effect of free radicals.

KEYWORDS: Gongronemalatifolium, Lasiantheraafricana, antioxidants, free radicals and DPPH.

INTRODUCTION

Free Radicals and related species have attracted a great deal of attention in recent years. They are mainly derived from oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS) and are generated in the body by various endogenous systems, exposure to different physiological conditions or pathophysiological states. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) play important roles in many biological processes and are involved in host defense (Eze *et al.*, 2000; Eze, 2006). Over production of these species such as hydroxyl radical (OH), hydrogen peroxide (H_2O_2), super oxide anions (O_2), and nitric oxide (NO), as well as peroxyl radical contributes to the immunopathology of a vast variety of conditions such as inflammatory diseases, cancer, atherosclerosis, diabetes mellitus, hypertension, AIDs and aging (Darley-Usmar *et al*., 1995). These reactive species are also implicated in food deterioration. Thus to sustain life, some enzymes in the human system like catalase, super-oxide dismutase (SOD), glutathione systems help to regulate and control the escalation of free radicals in the body, a situation known as oxidative stress.

Oxidative stress is a disturbance in the balance between the production of reactive oxygen species or free radicals and antioxidant defense, which may lead to tissue injury. Complementary to these enzymes and some plants species they have been found useful in controlling or regulating the over-production of these free radicals in the body (Manjo *et al.*, 2009). Recently, the roles of complementary and alternative medicine in the treatment of various acute and chronic diseases have been of great interest. For decades, the screening of medicinal plant materials for their therapeutic values has continued to represent potential sources of new effective medicines. Besides, evidence from epidemiological studies suggest that high consumption of fruits and vegetables is linked to reduced risk of developing most of the oxidative stress induced diseases (Dani *et al.*, 2008; Wasson *et al*., 2008; Atrooz, 2009). Example of such diseases includes cancer, diabetes mellitus, protein energy malnutrition (PEM), cataract, infections and other degenerative diseases and premature aging (Dani*et al*., 2008; Atwoz., 2009; Omoregie and Osagie, 2011; Dhanasekaran Ganapathy, 2011).

"Utazi" as G.*latifolium* is commonly called by the Ibibios and the Igbos of south-south part of Nigeria, is aclimbing shrub up to 5m long. The plant has a hallow stem with all parts soft, hairy, glabrous with woody base and roots containing latex. The plant has sharp-bitter and sweet taste and its leaves are widely used in Serra Leone, Ghana, Cameroon and Nigeria as a leafy vegetable, as a spice for sauces, soups, salads and locally brewedbeer. Phytochemical analysis of G.*latifolium* leaves reveals the isolation of several 17β marsdenin derivative (pregnane glycosides), as well as β-sitosterol, lupeny1 cinnamate, lupeny1 acetate, Lupeny1, essential oils and saponins.

G.*latifolium* is widely used in West Africa for medicinal and nutritional purposes: an infusion of the aerial parts is used to treat cough, intestinal worms, dysentery, dyspepsia and malaria. It is also taken as a tonic to treat loss of appetite. In Serra Leone an infusion or decoction of the stems with lime juice is taken as a purge to treat colic and stomach ache. In Senegal and Ghana the leaves are rubbed on the joints of small children to help them walk. The boiled fruits in soup are eaten as a laxative. A decoction of leaves or leafy stems is commonly taken to treat diabetes and high blood pressure. It is also taken for controlling weight gain in lactating women and overall health management. A cold maceration of the roots is taken as a remedy for asthma. The maceration of the leaves in alcohol is taken to treat viral hepatitis and as a general antimicrobial agent (Oboh *et al.,* 2009).

Lasianthera africana popularly known by the Ibibio and Efik as "Editan" is used by the people of southern Nigeria to prepare the popular 'Editan' soup and for treatment of some ailments. These communities have for several generations used these plants for medicinal and nutritional purpose and the leaves of this plant are used to treat bacteria skin infections, gonorrhea and abdominal disturbance. Studies on L. a*fricana* found that the plant contained tannins, anthraquinones, glycosides, reducing compounds. Crude aqueous and alcoholic extracts of the leaves were found to inhibit a number of test microorganisms excluding micrococcus and Brucella arbortus (Ebana *et al.,* 1995).

Vitamin C (Ascorbic acid) which is used as a standard for this work is well known and widely used antioxidant (Lawrence 2013). It is an electron donor, water-soluble antioxidant in human (Sebastian *et al.,* 2003). Its antioxidant effects have been demonstrated in many in-vitro experiments. Epidemiological studies show that diet high in fruit and vegetables are associated with lower risk of cardiovascular disease, stroke and cancer and with increased longevity (Sebastian *et al.,* 2003).

Reports abound on antioxidant activities of phytochemical and constituents of medicinal plants (e.g. Carotenoids, flavonoids, phenolics, vitamins C and E), these phytochemicals act as antioxidants (Oboh and Rocha 2008; Wasson *et al.,* 2008; Ebrahimzadeh *et al.,* 2009; Atrooz 2009; Omoregie and Osagie, 2011; Kasote *et al*., 2011). For instance, natural polyphenols from plant vegetables have been found to exert their beneficial effects by removing free radicals, chelating metal catalysts, activating antioxidant enzymes etc. (Atrooz *et al.,* 2009; Oboh *et al.,* 2009). Thus, emphasis will be laid on the antioxidant potentials of the two plants; *Gongronema latifolium* and *Lasianthera africana* which are widely consumed by the people of south-south part of Nigeria.

MATERIAL AND METHODS

Collection and Identification of Plant Materials

Fresh leaves of *Gongronema latifolium* and *Lasianthera africana* were purchased from Akpan-Andem Market in Uyo, Akwa Ibom State of Nigeria. The plants were authenticated by Dr (Mrs.) U. E. Eshiet, a plant Taxonomist of Botany and Ecological Department, Faculty of Sciences, University of Uyo, Nigeria. The plant leaves were deposited in the University of Uyo herbarium, Department of Pharmacognosy and Natural medicines. *Gongronema latifolium* was given voucher number which was No 9(a), while that of the Lasianthera *africana* was No. 36(b), the code was UUPH.

Preparation of Plant Extracts

The leaves of the two plants were rinsed with water to remove dirts and then cut into small pieces and then dried at room temperature. The dried leaves were pulverized mechanically using mortar and pestle. 109g of pulverized *G.latifolium* leaves was extracted by soaking in 1500ml of pure methanol for 72 hours at room temperature with intermittent shaking. For *L.africana*, the powdered leaves, 240g was soaked in 2500ml of pure methanol for 72 hours at room temperature with intermitted shaking. The mixtures were subsequently filtered at the end of the extraction period and concentrated in a water bath at temperature of 45-50^{o}C. The concentrate (marc) was stored in the refrigerator at -40^{o}C until used.

Antioxidant Assay Estimation of DPPH radical Scavenging Activity

Antioxidant activities of the methanol leaf extracts of *G.latifolium* and *L.africana* were measured as their ability to scavenging stable DPPH radicals. The scavenging activity of the plant extracts and standard vitamin C were determined using the modified Blois methods (Blois, 1958). Five different concentrations (500, 400, 300, 200 and 100ɥg/ml) of extracts were prepared in methanol using different test tubes.1 ml of 0.3Mm of freshly prepared DPPH solution in methanol was added to 2ml solution of each extract and standard vitamin C solution. The mixtures were allowed to react in the dark at room temperature for 30minutes. Thedegrees ofabsorbance of the resulting solutions were measures at 517nm using spectrophotometer against DPPH control containing only 2ml of methanol in place of the extracts (i.e 1 ml of 0.3 mM DPPH solution added to the 2 ml of methanol served as a negative control). Ascorbic acid prepared in the same concentrations as the test extracts was used as the reference standard for comparison. The degrees of absorbance were measured in triplicates and the scavenging activities in percentage were calculated. The percentage scavenging activity for each concentration represented the mean of five values obtained from triplicate determination. Percentage DPPH scavenging activity of the extracts and reference drug were determined using the formula: Percentage Scavenging activity=(Absorbance of Control-Absorbance of sample) x 100% Absorbance of control.

IC50 values obtained from the graphs plotted and the values denoted the concentration of sample required to scavenge 50% of the DPPH free radical.

1.3 Statistical Analysis

Data were expressed as means± Standard Error of Mean (SEM). The data analysis was done using the Graph pad Instant Computer Software Version 3.10. One- way analysis of variance (ANOVA) was used to compare the means of the five groups. P value> 0.05 was regarded as insignificant. Graph was plotted using Excel 2007 (Version 11.0). The IC50 values were determined from the graphs of scavenging activity against the different concentrations of the samples.

RESULTS

From the results, it was observed that; the absorbance decreased with increased concentration while the scavenging activity increased with increased concentration. (Table 1) The degrees of absorbance decreased with increased concentration while the scavenging activity increased with increased concentration. (Table 2) The absorbance decreased with increased concentration while the scavenging activity increased with increased concentration. (Table 3) The absorbance and DPPH scavenging of different concentrations of methanol extracts of the samples used and their IC50 are presented in the tables below:

Table 1: The Absorbances and DPPH Free Radical Scavenging Activities of Various Concentrations of Vitamin C tablet.

Concentration µG/ML	Absorbance at 517nM	Scavenging Activity (%)
100	0.074 0.073 0.074	93.74
200	0.072 0.071 0.074	93.85
300	0.070 0.070 0.072	93.96
400	0.070 0.070 0.072	94.05
500	0.068 0.071 0.071	94.05

Table 2: The Absorbances and DPPH Free Radical Scavenging Activities of Various Concentrations of *Gongronema latifolium*.

Concentration (µG/ML)	Absorbances at 517nM	Scavenging Activity (%)
100	0.338 0.342 0.337	71.17
200	0.237 0.236 0.238	79.85
300	0.210 0.209 0.208	82.23
400	0.173 0.172 0.173	85.32
500	0.162 0.162 0.163	86.19

Table 3.3: The Absorbances and DPPH Free Radical Scavenging Activities of Various Concentrations of *lasianthera Africana.*

Concentration (µG/ML)	Absorbances at 517nM	Scavenging Activity (%)
100	0.381 0.382 0.381	67.57
200	0.308 0.307 0.309	73.81
300	0.235 0.234 0.238	79.96
400	0.206 0.208 0.208	82.37
500	0.200 0.199 0.204	82. 91

Table 4: The Scavenging activities and IC_{50} of Vitamin C tablet, *Gongronema latifolium and lasianthera africana.*

	Sample	Concentration	Scavenging Activities (%)	Mean Scavenging Activity(%)	IC_{50} (µg/ml)
1.	Vitamin C table	100.0 200.0 300.0 400.0 500.0	93.74 ±0.0461 93.85 ±0.1266 93.96 ±0.900 94.05 ±0.1750 94.05 ±0.1501	83.93±0.12	50.0
2.	*Gongronema latifolium*	100.0 200.0 300.0 400.0 500.0	71.17 ±0.2230 79.85 ±0.0850 82.23 ±0.0850 85.32 ±0.0461 86.19 ±0.0461	80.95±0.09	70.0
3.	*Lasianthera africana*	100.0 200.0 300.0 400.0 500.0	67.57 ±0.0461 73.81 ±0.0850 79.96 ±0.1777 82.37 ±0.0981 82.91 ±0.2267	77.32±0.13	75.0

Absorbance of the black (negative control) at 517.0mm = 1.176
The scavenging activities for each concentration are mean of triplicate determination ± SEM
MSA of *Gongronema latifolium* versus MSA of Vitamin C tablet – NSD (P>0.05)
MSA of *Lasianthera africana* versus MSA of Vitamin C tablet – NSD (P>0.05)
MSA = Mean Scavenging activity
NSD = No Significant Difference

The graphs showing the scavenging activity of each of the sample are given below:

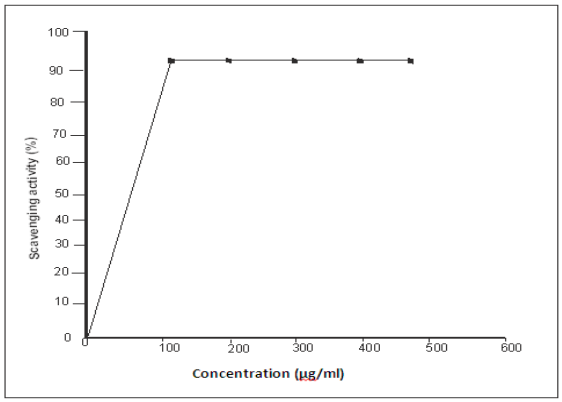

Figure 1: Scavenging Activity of Vitamin C Tablet.

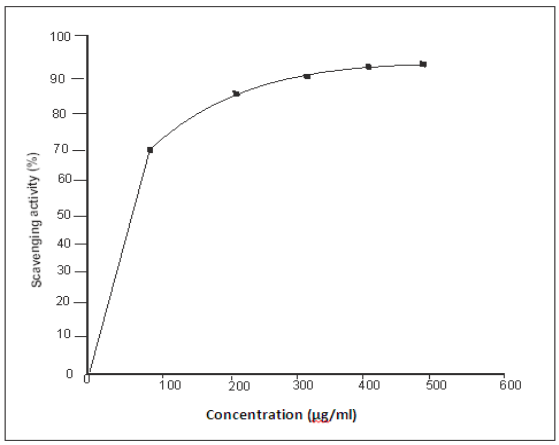

Figure 2: Scavenging Activity of *Gongronema latifolium.*

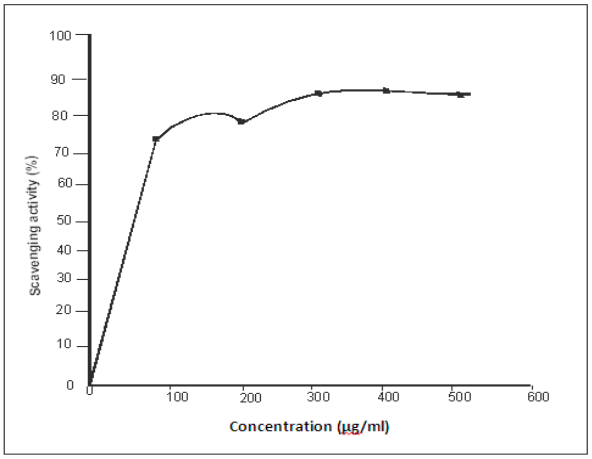

Figure 3: Scavenging Activity of Lasianthera *africana.*

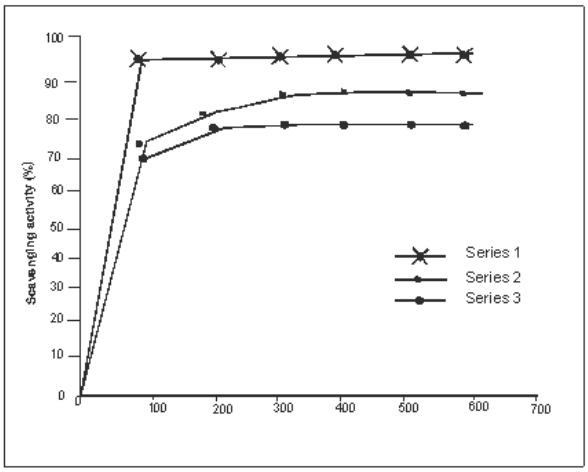

Figure 3.4: Scavenging Activities of *Gongronema latifolium and Lasianthera Africana.*

Compared to Vitamin C Tablet

Series 1 → % Scavenging Activity of Vitamin C tablet

Series 2 → % Scavenging Activity of *Gongronema latifolium*

Series 3 → % Scavenging Activity of *Lasianthera africana*

4.0 DISCUSSION

From this work, it was discovered that the two widely used medicinal plants; *Gongronema latifolium* and *Lasianthera africana* have antioxidant activity expressed as IC_{50}. These activities were found to increase as concentration increased. For example, the antioxidant activities exhibited by 200µg/ml were higher than the activities at 100µg/ml. Also, the activities at 50µg/ml were higher than the activities at 400µg/ml. that is to say, with increased concentrations, the DPPH radical scavenging activity increased. The standard, Vitamin C was found to possessed high mean scavenging activity which was 93.93 + 0.12 as compare to that of the extracts which were 80.95 + 0.09 for *Gongronema latifolium* and 77.32 +0.13 for *Lasianthera africana*. Comparing the mean scavenging activities of these extracts with that of the standard vitamin C, the differences observed were not significant ($p>0.05$). That is for the mean scavenging activities of *Gongronema latifolium* (80.95 +0.09), compare to that Vitamin C tablet (93.93 + 0.12) no significant different (P?0.05) was observed. Also, there was no significant difference ($P>0.05$) between the mean scavenging activity of *Lasianthera africana* (77.32+0.13) and that of vitamin C tablet. It was also observed that *Gongronema latifolium* possessed higher antioxidant potential than *Lasianthera africana* but vitamin C exhibited the highest.

Considering the IC_{50}, the standard ascorbic acid tablet had the lowest IC_{50} which was 50.0µg/ml. The IC_{50} values of *Gongronema latifolium* and *Lasianthera africana* were 70.0µg/ml and 75.0µg/ml respectively. This is an important parameter which portrays the potencies of the samples evaluated and its expresses the amount of antioxidant required to decrease the DPPH radical concentration by 50% (Chanda *et al.*, 2011). A low IC_{50} corresponds with a higher antioxidant power. Comparing the IC_{50} of the two extracts with that of vitamin C, it can be inferred that the potency of vitamin C tablets as free radical scavenger is greater than that the *Gongronema latifolium* and *Lasianthera africana*but the plants also possess significant potencies.

From various studies, it has been observed that phenolic substances and flavonoids are associated with antioxidant activities of medicinal herbs and they play important role in stabilizing lipid peroxidation (Joyce, 1987, Yeh-Lin *et al.*, 2012). This is by absorbing and neutralizing free radical, quenching singlet and triplet oxygen, and decomposing peroxides (Dorman *et al.*, 2003). Phenolic compounds are classified as simple phenols and polyphenols based on the number of phenol units in the molecule (Amorati and Valgimigli, 2012). The antioxidant potential of the phenolic compounds is believed to be conferred on them by their hydroxyl group (-OH), which is bonded directly to an aromatic hydrocarbon (phenyl) ring. This makes them donate electrons easily to electron seeking free radicals, thus down regulating their menace in living cells. The antioxidant capacity of these phenolic compounds is mainly attributed to their redox potential (Demiray *et al.*, 2009; Premanath and Lakshmideri, 2010). In many phytochemical studies, *Gongronema latifolium* and *Lasianthera africana* have been found to contain high amount of flavonoid and phenolic compounds. These phytochemicals are responsible for their activities.

CONCLUSION

Antioxidant effects of these plants explain their wide acceptability in folkloric medicines to prevent, manage, and slow down the progression of various oxidative stresses related diseases. The result of this study shows that the widely used *Gongronema latifolium* and *Lasianthera africana* have appreciably potent antioxidant

activities that cannot be neglected. The study reveals that these plants are reservoirs of natural antioxidants which can be utilized nutritionally and medicinally. Hence, increased domestication of these plants could provide raw materials which are affordable and easily accessible. Increased consumption of these vegetables is thereby recommended to help mitigate against deleterious effect of reactive species known as free radicals.

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