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

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Abstract

The study evaluated the phenolic content and antioxidant capacities of *Lasianthera africana* leave stem and root extracts which 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$_2$O$_2$) and 2,2-azinobis (3-ethylbenzothiazol-6-sulfonic acid) diammomium 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 hydroxyanisole (BHA) and 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$_2$O$_2$ 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$_2^-$), peroxyanion (ONOO$^-$), peroxy radical (ROO$^.$), hydrogen peroxide (H$_2$O$_2$), nitric oxide (NO$^.$) and hydroxyl radical (OH$^.$). 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, canceroma, inflammations, asthma, cardiac arrest, diabetes mellitus, fibroids, parasitic infections, ulcers and diabetes. Its fruits are also used for treatment of asthma, hypertension, skin diseases and wounds healing$^{3,4}$. *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$^{3,4}$. *Lasianthera africana* leaves are reported to posses antiulcerogenic$^{14}$, antimalarial$^{15}$ and antimicrobial$^{16,17}$. The total phenolics and flavonoids content of the leaves was also reported$^{18}$.

There is fart 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 thiocyanate 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. 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):

\[ A = 0.1216 Z, \quad R^2 = 0.936512 \]

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. 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% AlCl3 (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 regarding 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:

\[ A = 0.255 Z, \quad R^2 = 0.9812 \]

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, 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. was adopted to ascertain the NO scavenging capacity of L. africana leave, stem and root extracts. A 2 mL solution of 10 mM Na2[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% H3PO4, 1% sulphanalimade and 0.1% Naphthylethenediamine 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:

\[ \% \text{Inhibition} = \left( \frac{\text{ABS}_c - \text{ABS}_e}{\text{ABS}_c} \right) \times 100 \]

Where ABSc was the absorbance of NO radical + methanol; ABSe was the absorbance of NO radical + sample or standard of test.

Determination of DPPH scavenging activity: The method of Shen et al. 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 λ = 517 nm. The capacity of the plant extracts to abstract the DPPH free radical was mathematically estimated by the following equation:

\[ \% \text{Inhibition} = \left( \frac{\text{ABS}_c - \text{ABS}_e}{\text{ABS}_c} \right) \times 100 \]

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

Determination of ABTS scavenging capacity: The procedure mapped out by Re et al. was adopted to determine the scavenging capacities of the plant extracts against ABTS' *
radical cation. Two stock solutions; 7 mM 2, 2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid), (ABTS) and 2.4 mM potassium persulphate (K₂S₂O₈), were prepared and mixed in equal proportions. The mixture was kept in the dark cupboard for 12 hours at ambient temperature. After this, ABTS⁺ 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⁺ radical separately and absorbance was taken after 7 mins. The amount of ABTS⁺ cation inhibited by the extracts as compared to the reference standard rutin is represented by the equation:

\[
\text{\% ABTS}^+ \text{ Scavenging Capacity } = \left( \frac{\text{ABS}_c - \text{ABS}_s}{\text{ABS}_c} \right) \times 100
\]

Where ABSₖ was the absorbance of ABTS⁺ radical + methanol; ABSₛ was the absorbance of ABTS⁺ 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:

\[
\text{\% [H₂O₂] Scavenged } = \left( \frac{\text{ABS}_c - \text{ABS}_s}{\text{ABS}_c} \right) \times 100
\]

Where ABSₖ was the absorbance of H₂O₂ + methanol; ABSₛ was the absorbance of H₂O₂ + 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 a vasoconstriction effect on small superficial vessels; antimicrobials, antioxidants, anti-inflammatory, antiviral, anti-diarrhoeal, antimutagenic and chemopreventive²⁷. Phytochemicals 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.³⁰ 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](image)

**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 Fe³⁺ to Fe²⁺. 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 IC₅₀ 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)\(_5\)NO]) 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%, 51 and 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.
Nitric Oxide scavenging activities of the ethanol extracts of *L. africana* leaves stem and root in comparison to rutin

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>DPPH IC$_{50}$</th>
<th>DPPH R$^2$</th>
<th>NO IC$_{50}$</th>
<th>NO R$^2$</th>
<th>H$_2$O$<em>2$ IC$</em>{50}$</th>
<th>H$_2$O$_2$ R$^2$</th>
<th>ABTS IC$_{50}$</th>
<th>ABTS R$^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutin</td>
<td>0.26</td>
<td>95.4</td>
<td>0.28</td>
<td>89.2</td>
<td>0.29</td>
<td>73.9</td>
<td>0.29</td>
<td>89.4</td>
</tr>
<tr>
<td><em>L. africana</em> leaves</td>
<td>0.30</td>
<td>96.9</td>
<td>0.29</td>
<td>91.5</td>
<td>0.32</td>
<td>80.1</td>
<td>0.30</td>
<td>93</td>
</tr>
<tr>
<td><em>L. africana</em> stems</td>
<td>0.31</td>
<td>96.6</td>
<td>0.29</td>
<td>92.3</td>
<td>0.31</td>
<td>81.5</td>
<td>0.31</td>
<td>92.4</td>
</tr>
<tr>
<td><em>L. africana</em> roots</td>
<td>0.30</td>
<td>97.7</td>
<td>0.27</td>
<td>94.5</td>
<td>0.31</td>
<td>78.8</td>
<td>0.30</td>
<td>92.6</td>
</tr>
<tr>
<td>BHT</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>0.33</td>
<td>80.1</td>
<td>n/d</td>
<td>n/d</td>
</tr>
<tr>
<td>Vit. C</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
<td>n/d</td>
</tr>
</tbody>
</table>

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$_2$O$_2$ 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$_2$O$_2$ by the plant’s ethanol extracts were observed in a concentration dependent manner. At 0.3 mg/mL, the percentage decomposition of H$_2$O$_2$ 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$_2$O$_2$ and thereby rendering it ineffective by forming water$^{33}$. The scavenging effect of different extracts on H$_2$O$_2$ 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 activity. The scavenging potential of the three extracts of *L. africana* on ABTS \(^{\cdot+}\) 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*\(^{30,34}\).
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.

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