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(SHORT COMMUNICATION)

Free radical scavenging activity of two edible vegetables from the Niger delta region of Nigeria

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Abstract

Oxidative stress and diseases that have their aetiology and pathophysiology in reactive oxygen species can be prevented by medicinal plants and herbs. We undertook in this study, to investigate the free radical scavenging activity of dichloromethane (DCM) and ethanol (ETOH) extracts of *Lasianthera africana* and *Gongronema latifolium in vitro* using 2, 2, diphenyl-1-picrylhydrazine (DPPH). The leaves of both plants were obtained locally, washed, dried and pulverized using mechanical grinder. The powdered materials were defatted using n-hexane and subsequently extracted with DCM and thereafter with ETOH. The DPPH scavenging potentials of the extracts were then evaluated. The percentage inhibition of the DCM and ETOH extracts were; 46.2 and 80% respectively for *L. africana* and 37.2 and 82.6 respectively for *G. latifolium.* The standard substance (quercetin) produced 98.4 per cent inhibition at 1 mg/mL. The IC_{50} for the *L. africana* were; 2.017 and 3.256 mg/mL; DCM and ETOH extracts respectively. While that of *G. latifolium* were 1.495 and 1.116 mg/mL respectively for DCM and ETOH extracts. The standard substance produced IC_{50} of 0.55 mg/mL. The DPPH inhibitory activity was found to be prominent with the ETOH extracts of both plants. The result from this study validates the use of the leaves of both plants as supplements to improve health conditions and quality of life in general.

Keywords: *Gongronema latifolium*; *Lasianthera africana*; Free radical; Phytochemical

1. Introduction

Reactive oxygen species are responsible for oxidative stress and so many other degenerative diseases such as diabetes, inflammatory and cardiovascular disorders. Medicinal plants and herbs are veritable sources of natural antioxidants that can be used in the chemoprevention of such disorders [1]. Plant secondary metabolites such as flavonoids are responsible for the properties possessed by medicinal plants. Some organic and inorganic compounds such as coumarins, phenolic acids and antioxidants micronutrients; Cu, Mg, Zn also contribute to the efficacy of most medicinal plants [1]. The Niger delta is home to some important herbs in Nigeria that are consumed by the natives either for nutritive or therapeutic purposes. *Lasianthera africana* and *Gongronema latifolium* are two of such vegetable herbs.

L. africana, (BEAUV), family; Icacinaceae, is one of the top six commonly consumed green leafy vegetables by Efik and Ibibio ethnic groups of Nigeria [2]. It is locally called "editan" by Efiks and Ibibios. It is a perennial, glabrous, shrub that reaches a height of 61-136 cm [3]. Four local varieties characterized by their taste, leaf colour and ecological distribution are known among the Ibibios [4]. The varieties are "afia" (white variety) "obubit" (black variety), "idim" (riverine variety) and "Akai" (forest variety). The use of *L. africana* predates modern history. It is used in the preparation of soups and as decoctions, for the treatment of various diseases [5]. The leaf of *L. africana* is reported to be rich in phytochemicals of nutritional and medicinal importance [6]. Preliminary screening of the leaves showed the

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presence of some secondary metabolites such as flavonoids, alkaloids, anthraquinones, saponins and tannins in all the four ethno varieties [4]. The leaf of *L. africana* is commonly used for nutritional purpose and medicinally in the treatment of fever, malaria and inflammatory diseases.

G. latifolium (Benth Hook), belongs to the family Asclepiadaceae and is a tropical rainforest plant primarily used as spice and vegetable in food [7]. *Gongronema* is a large genus that includes several major cultivated species including *Gongronema latifolium, Gongronema bracteolatum, Gongronema obscurum, Gongronema curtisii, Gongronema wrayi, Gongronema angolense. G. latifolium* is listed among the twenty-eight medicinally important vegetables of south west Nigeria [8] and also one of the aromatic plants of medicinal importance from Nigeria [9]. *G. latifolium* is known as "utazi" and "arokeke" in the Eastern and Western Nigeria respectively. It is an important medicinal plant, spice and vegetable. Some pharmacological test conducted in the past have indicated promising hypoglycaemic activities and also interesting antibacterial, anti-inflammatory, hepatoprotective, antiplasmodial, anti-asthmatic, anti-ulcer, analgesic, antipyretic and antisickling activity [10]. Preliminary phytochemical screening of *G. latifolium* leaves revealed the presence of alkaloids, saponin, tannins, flavonoids and glycosides, [11].

2. Material and methods

2.1. Chemicals

All solvents used in the experiment (n-hexane, dichloromethane, ethyl acetate, absolute ethanol, methanol) were purchased from Loba Chemic Pvt. Ltd (India). Quercetin, 2, 2-diphenyl-1-picrylhydrazine (DPPH), gallic acid, Folin ciocalteu's reagent and sodium carbonate were purchased from sigma chemicals (USA). All other chemicals and reagents used were of analytical grade.

2.2. Plant materials

The leaves of *Lasianthera Africana* and *Gongronema latifolium* were purchased from Agudama market, Yenagoa town, Bayelsa State (South of Nigeria). They were identified and authenticated by Dr. T. Oladele, Department of Pharmacognosy and herbal medicine, Faculty of Pharmacy, Niger Delta University. The leaves were oven dried at 40 °C and pulverized into fine powder using a mechanical grinder.

2.3. Extraction

The powdered materials (400 g) were extracted by macerating with n-hexane followed by dichloromethane and then absolute ethanol respectively with occasional shaking and allowed to stand, followed by filtration at room temperature. Each extraction was done three times to ensure complete extraction. The filtrate from each respective extract of n-Hexane, dichloromethane and absolute ethanol were concentrated by evaporating on the water bath set at 35 °C.

2.4. Free radical scavenging activity

The electron donating ability of the extracts was measured by bleaching of the purple coloured solution of 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) according to the method of Ebrahimzadeh *et al* [13, 14, 15] with a slight modification, a 0.5 mM DPPH solution in methanol was prepared and then, 1 mL of this solution was mixed with 3 mL of the extract solution, concentrations (0.2 – 1 mg/mL). The mixture was shaken and left to stand in a dark cupboard at room temperature for 30 min and the absorbance of the resulting solution was measured at 517 nm. The percentage (%) DPPH radical scavenging was calculated using the following formula;

$$\%\ \text{DPPH scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_o and A_1 are the absorbance of the blank and sample respectively, after 30 min. The antiradical activity was carried out in triplicate per treatment and the IC_{50} (concentration required to cause a 50% DPPH inhibition) was estimated. Quercetin was used as the positive control.

2.5. Statistical analysis

Statistical analysis was performed with graph pad prism 7 demo. Differences were tested for significance by linear regression procedure, using a significance level of $P \leq 0.05$.

3. Results and discussion

Table 1 Percentage DPPH scavenging effect of dichloromethane and ethanol extracts of the leaf of *L. africana* and quercetin

Concentration (mg/mL)	Percentage of inhibition (%)		
	DCM extract	ETOH extract	Quercetin
1	46.20	80.00	98.40
0.8	42.10	79.70	98.30
0.6	39.30	78.70	98.20
0.4	37.90	77.90	97.30
0.2	30.60	77.30	93.00

Table 2 Percentage inhibition for dichloromethane and ethanolic extract of the leaves of *G. latifolium* and quercetin

Concentration (mg/mL)	Percentage of inhibition (%)		
	DCM extract	ETOH extract	Quercetin
1	37.21	82.64	98.42
0.8	30.94	80.94	98.31
0.6	20.31	76.25	98.17
0.4	18.94	77.96	97.17
0.2	11.30	70.43	93.03

3.1. DPPH radical scavenging activity

The free radical scavenging activity of the extracts of the leaf of *L. africana* and *G. latifolium* was determined using DPPH method, a stable free radical which is widely used to assess the radical scavenging activity of antioxidant compounds [12, 13, 14]. The antioxidant effect is proportional to the decolourization and disappearance of the purple colour of DPPH. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples [15]. It was found (Table 1 and 2), that the increase in concentration of the extract of *L. Africana, G. latifolium* and the standard quercetin favours the radical scavenging activity. This means that, the higher the concentration, the more the free radical scavenging activity. High absorbance is an indication of high concentration of formed peroxides-Therefore low concentration indicates high antioxidant activity [16]. The high phenolic and flavonoid contents of the leaf of *L. africana* and *G. latifolium* are responsible for high antioxidant activity of the leaf. Phenols and polyphenols compounds such as flavonoids found in food products derived from plant sources such as *Lasianthera africana* and *G. latifolium* have shown to possess significant antioxidant activities [17].

From linear regression analysis, the (DCM) and (ETOH) extracts of *L. africana* indicated; $y = 3.6x + 76.56$, $r^2 = 0.9803$ and $Y = 17.7x + 28.6$, $r^2 = 0.9417$ respectively. That of *G. latifolium* is; $y = 31.91x + 4.5\ 94$, $r^2 = 0.96$ and $y = 13.7x + 69.42$, $r^2 = 0.83$ for DCM and ETOH respectively. While quercetin indicated; $y = 5.9x + 93.5$, $r^2 = 0.6577$. The IC_{50} (concentration required to cause a 50% DPPH inhibition) for the leaf extracts of *L. africana* was obtained from linear regression curve as 2.017 and 3.256 mg/mL; DCM and ETOH extracts respectively. While that of *G. latifolium*, were 1.495 and 1.116 mg/mL respectively. The standard substance; quercetin produced 0.55 mg/mL as its IC_{50}. Low IC_{50} indicates greater antioxidant activity [18]. This means that the standard substance; quercetin has more potential antioxidant activity than the leaf extracts. It is shown that only flavonoids with certain structure and particularly hydroxyl group in certain positions in their molecule can act as proton donating and show radical scavenging activity. Similarly, the extracts contain complex mixtures of many different compounds with distinct activities [19, 20]. The antioxidant activities of putative antioxidants have been attributed to various mechanisms; among these are prevention of chain initiation, binding of transition ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction and radical scavenging [21, 22]. In addition, it has been reported that the free –OH groups in phenolic compounds are mainly

responsible for antioxidant activity [23]. Thus, these results may suggest that DPPH scavenging activity is an indication of potential antioxidant activity for the leaf extracts of *L. africana* and *G. latifolium.*

4. Conclusion

In this study, the free radical scavenging and antioxidant activity was found to be more in the ETOH extracts compared to the DCM extracts. This shows that the antiradical agents are predominantly in the polar extract; which in no doubt should be the case because the polar phase should contain more of the phenolic compounds that may be responsible for the radical scavenging activity. The findings from this study has provided a rationale for the ethnobotanical use of the plants to improve health conditions; further work should be carried out to isolate chemical constituents responsible for their activity.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest in this work.

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