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Research Article

Studies on *in vitro* Antioxidant activities, Mineral composition and Phytochemical screening of *Gnetum africanum* leaves

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Abstract: *Gnetum africanum*, a common soup vegetable in Nigeria is also been used in the treatment of enlarged spleen, sore throats, deduction of pains of child-birth, antidotes to some forms of poison and snake bite. This study is to ascertain phytochemicals and minerals present in the leaves of *Gnetum africanum* in addition to *in vitro* antioxidant activities using standard procedures. The result of the phytochemicals present revealed the presence of flavonoids, saponins, tannins, alkaloids while the minerals present in mg/100g includes: calcium (423.20), magnesium (112.81), phosphate (83.44), zinc (2.53), sodium (37.82), potassium (86.10), copper (1.00), Iron (11.67) and chromium (0.52). *In vitro* antioxidant activities using reducing power activity and DPPH radical scavenging assays showed that as concentration of *Gnetum africanum* increases from 0.2mg/ml to 1.0mg/ml, reducing power ability and radical scavenging activity also increases. In conclusion, the presence of bioactive agents such as flavonoids as well as radical scavenging ability of *Gnetum africanum* gives credence to its usage in herbal medicine for treatment of various ailments**.**
Keywords: 2, 2-diphenyl-1-picrylhydrazyl, *Gnetum africanum*, in vitro, Leaves, Minerals, phytochemicals.

INTRODUCTION

Gnetum africanum (family, Gnetacea) locally called Afang (Efik), Okazi (Ibo) and Eru (Cameroun) is a wild climbing plant with dark green leaves tasting sweet or slightly bitter when eaten and is often listed in many continental restaurant menu. *Gnetum africanum* leaves are widely consumed in South Eastern Nigeria due to its palatability and taste. It is now eaten as a vegetable salad when mixed with palm oil. The leaves of the plant are also used in Nigeria herbal medicine for the treatment of enlarged spleen, sore throats and as a cathartic [1]. Literature reveals that both the leaf and the seed possesses medicinal efficacy in the treatment of pains of child-birth, antidotes to some forms of poison and snake bite. The seeds are specially used as fungicide for dressing fresh and septic wounds. It is also chewed raw in the management of excessive urination by infantile diabetic patients in traditional medicine practice [2-4]. The aim of this study is to screen for phytochemicals and minerals as well as *in vitro* antioxidant activities of *Gnetum africanum* leaves.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Materials

The fresh leaves *Gnetum africanum* were collected from a local farm in south eastern part of Nigeria. Identification and authentication were carried out after which the leaves were washed and air dried at room temperature for fourteen (14) days. They were grounded into fine powder using an electric blender and stored in a cool dry container until use for analysis.

Phytochemical Analysis

Qualitative phytochemical screening using standard methods as described [5-9] were carried out.

Mineral Analysis

Mineral analysis was carried out using Atomic Absorption Spectrophotometer (AAS) as previously done by Usunobun & Okolie [10-11].

Determination of Reducing Power Ability

The reducing power ability of *Gnetum africanum* leaves was carried out using the reducing power method as described by Aiyegoro & Okoh [12]. A mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of stock *Gnetum africanum* leaves filtrate (0.2-1.0 mg/ml) prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), followed by centrifugation at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was measured at 700 nm against reagent blank sample. Increased absorbance of the reaction mixture indicates higher reducing power of *Gnetum africanum* leaves.

2,2-Diphenyl-1-Picrylhydrazyl (Dpph) Radical Scavenging Ability

The DPPH method according to Liyana-Pathiana & Shahidi [13] was used for the determination of DPPH free radical scavenging activity of the *Gnetum africanum* leaves as follows: DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of stock *Gnetum africanum* leaves filtrate ranging in concentration from 0.2 - 1.0 mg/ml. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging ability was calculated using the equation:

DPPH scavenging activity (%) =

$$[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$$

Where: $Abs_{control}$ is the absorbance of DPPH + methanol and Abs_{sample} is the absorbance of DPPH radical + sample (sample or standard).

Statistical Analysis

Data obtained from this study were expressed as mean value ± standard deviation.

RESULTS AND DISCUSSION

The result of the photochemical evaluation is shown in Table 1. It revealed the presence of alkaloids, tannins, saponins and flavonoids and the absence of steroid and glycoside. The preliminary phytochemical analysis on the powdered leaves of *Gnetum africanum* indicated the presence of Saponin, Tannins, Flavonoids and Alkaloids. These bioactive constituents may be responsible for its ethnomedicinal effects. Alkaloids action are felt in the automatic nervous system, blood vessels, respiratory system, gastrointestinal tract. In addition, alkaloids are anti-pasmodic, analgesic and also have bactericidal effects. Tannins are well known for their anti-oxidant and anti-microbial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and diuretics properties. Saponin lower the cholesterol level, have anti-diabetic and anti-carcinogenic properties. In addition, saponins are expectorants, cough suppressant and for haemolytic activities. Flavonoids are significantly recognised for their anti-oxidant, anti-carcinogenic and anti-microbial and anti-tumour properties [14].

Table 1: Phytochemical screening of *Gnetum africanum* leaves

Phytochemical	*Gnetum africanum*
Flavonoid	Present
Saponin	Present
Alkaloid	Present
Tannin	Present
Anthraquinone	Absent
Steroid	Absent
Glycoside	Absent

The mineral composition of *Gnetum africanum* leaves in mg/100g as shown in table 2 includes: calcium (423.20), magnesium (112.81), phosphate (83.44), zinc (2.53), sodium (37.82), potassium (86.10), copper (1.00), Iron (11.67) and chromium (0.52). Minerals plays an important in human nutrition for instance enzymatic activities and electrolyte balance of blood fluid are related to adequacy of Na, K, Mg and Zn. Potassium is very important in maintaining the body fluid volume and osmotic equilibrium. Calcium deficiency leads to syndrome like rickets and calcification of bones. Magnesium is essential to all living cells and plays a major role in the functions of important biological polyphosphate compounds like ATP, DNA and RNA. Also iron is another component of *Gnetum africanum* which is important in a number of physiologic processes as a constituent of some enzymes and activation of other enzymes [15]. Zinc is involved in normal functioning of immune system [16]. Calcium content of *Gnetum africanum* (423.20mg/100g) is high compared to 128.30mg/100g of *Pterocarpus mildbraedii* [17] and 295mg/100g of *Celosia argentea* [18] but low compared to 1118.30mg/100g of *Annona muricata* and 1264.18mg/100g of *Vernonia amygdalina* [10-11]. Zinc content of *Gnetum africanum* (2.53mg/100g) compared favorably with 2.51mg/100g of *Pterocarpus mildbraedii* [17] but low compared to 5.42mg/100g of *Celosia argentea* [18] and high compared to 0.83mg/100g of *Annona muricata* and 1.42mg/100g of *Vernonia amygdalina* [10-11]. Sodium content of *Gnetum africanum* (37.82mg/100mg) compared favorably with 48.31mg/100g of *Vernonia amygdalina* [11] but low compared to 69.49mg/100g of *Annona muricata* [10], 60.32mg/100g of *Pterocarpus mildbraedii* [17] and 71.32mg/100g of *Celosia argentea* [18]. Potassium content of *Gnetum africanum* (86.10mg/100g) is low compared to 104.85mg/100g of *Pterocarpus mildbraedii* [17], 128.33mg/100g of *Celosia argentea* [18] but high when compared to 36.31mg/100g of *Annona muricata* and 62.79mg/100g *of Vernonia amygdalina* [10-11]. Copper content of *Gnetum africanum* (1.00mg/100mg) compared favourably with 0.95mg/100g of *Pterocarpus mildbraedii* [17], 1.95mg/100g of *Vernonia amygdalina* and 1.42mg/100g of *Annona muricata* reported by Usunobun & Okolie [10-11] but low compared to 2.18mg/100g of *Celosia argentea* (18). Iron content of *Gnetum africanum* (11.67mg/100g) compared favorably with 14.64mg/100g of *Pterocarpus mildbraedii* [17], 13.90mg/100g of *Annona muricata* [10] but low compared to 32.20mg/100g of *Vernonia amygdalina* [11] and 35.16mg/100g of *Celosia argentea* [18].

Table 2: Mineral composition of *Gnetum africanum* leaves in mg/100g

Minerals	*Gnetum africanum* (mg/100g)
Calcium	423.20±3.01
Magnesium	112.80±0.99
Potassium	86.10±0.84
Phosphate	83.44±0.81
Sodium	37.82±0.64
Iron	11.67±0.73
Zinc	2.53±0.33
Copper	1.00±0.99
Chromium	0.52±0.10

Values are means ± SD for 2 determinations

The result of reducing power ability of *Gnetum africanum* showed that as the concentration increases, the absorbance increases, thus the reducing power increases. In otherwords, the reducing power as shown in figure 1 below is dose-dependent as the reducing power was at its peak at 1.0mg/ml.

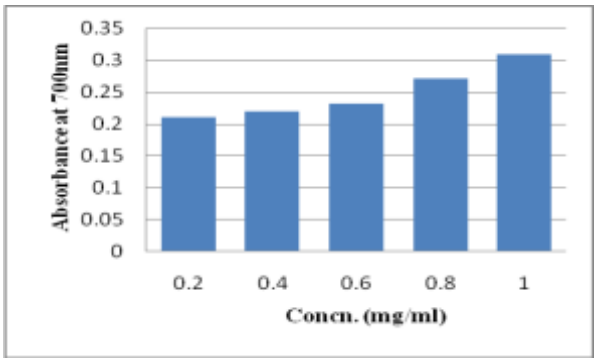

Fig-1: Reducing power ability of *Gnetum africanum* leaves

Gnetum africanum leaves evoked a concentration - dependent increase in percentage antioxidant activity in DPPH spectrophotometric assay. The highest concentration of the leaves (1.0 mg/ml) gave an antioxidant activity of 84.01% which is comparable to the effect (88.60%) of the same concentration of ascorbic acid, the reference antioxidant as shown in figure 2.

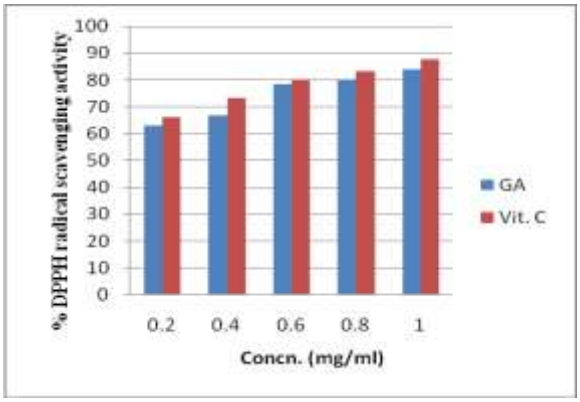

Fig-2: DPPH radical scavenging activity of *Gnetum africanum* (GA) leaves

The *in vitro* DPPH spectrophotometric and reducing power assays helps to determine antioxidant potential of samples. DPPH is a dark colored crystalline powder composed of stable free radical molecules [19]. From the result of DPPH spectrophotometric and reducing power assays, *Gnetum africanum* possess concentration dependent antioxidant activity comparable to that of ascorbic acid.

CONCLUSION

Conclusively, with the present policy on poverty alleviation in developing countries such as Nigeria, it is hoped that *Gnetum africanum* leaves could be explored as potential sources of drug production.

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