

Analysis of phytochemicals, minerals and *in vitro* antioxidant activities of *Gongronema latifolium* leaves

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ABSTRACT

Background study: *Gongronema latifolium* is primarily used as spice and vegetable as well as a herb in traditional medicine in the treatment of malaria, diabetes and hypertension. This study is aimed at providing *in vitro* laboratory knowledge on *Gongronema latifolium* leaves.

Methods: Minerals were analyzed using Atomic Absorption Spectrophotometer while phyto-nutrients were screened using standard laboratory procedures. 2,2-diphenyl-1-picrylhydrazyl (DPPH)-radical scavenging and reducing power activities were determined spectrophotometrically.

Results: Results showed that *Gongronema latifolium* leaves contains flavonoids, tannins, saponins, alkaloids, terpenoids etc as well as minerals such as calcium (333.40mg/100g), magnesium (38.90mg/100g), potassium (83.69mg/100g), sodium (31.02mg/100g), phosphate (51.13mg/100g), iron (4.88mg/100g), zinc (2.19mg/100g) and chromium (0.98mg/100g). DPPH-radical scavenging and reducing power activities of *Gongronema latifolium* leaves showed the plant be concentration dependent as free radical scavenging activity increases as concentration increases from 0.2-1.0mg/ml.

Conclusion: The presence of bioactive agents, minerals and free radical scavenging potentials of *Gongronema latifolium* leaves makes it a beneficial medicinal plant.

Keywords: 2,2-diphenyl-1-picrylhydrazyl, *Gongronema latifolium*, minerals, phytochemicals, reducing power

INTRODUCTION

Gongronema latifolium of the family Asclepiadaceae, locally known as arokeke by the Yoruba and utazi by the Igbos is a tropical rainforest plant primarily used as spice and vegetable in the traditional folk practice [1]. Traditionally, the southern part of Nigeria uses this herb in the treatment of malaria, diabetes and hypertension as well as a laxative. A maceration of the leaves in alcohol is taken to treat bilh.arzia, viral hepatitis and as a general antimicrobial agent [2]. It is also taken as a tonic to treat loss of appetite [3]. Asthma patients chew fresh leaves to relieve wheezing [4] and a decoction of the roots, combined with other plant species, is taken to treat sickle cell anaemia. Nwanjo [5] reported the anti lipid peroxidative activity of the plant. The objective of this study is to provide *in vitro* information of the leaves of *Gongronema latifolium* and to create awareness of the health benefits.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant materials

The fresh leaves *Gongronema latifolium* 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 [6-10] were carried out.

Mineral analysis

Mineral analysis was carried out using Atomic Absorption Spectrophotometer (AAS) as previously done by Usunobun and Okolie [11-12].

Determination of reducing power ability

The reducing power activity of *Gongronema latifolium* leaves was carried out using the reducing power method as described by Aiyegoro and Okoh [13]. 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 *Gongronema latifolium* 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 *Gongronema latifolium* leaves.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability

The DPPH method according to Liyana-Pathiana and Shahidi [14] was used for the determination of DPPH free radical scavenging activity of the *Gongronema latifolium* leaves as follows: DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of stock *Gongronema latifolium* leaves filtrate ranging in concentration from 0.2 to 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 \pm standard deviation.

RESULTS

Phytochemical screening of *Gongronema latifolium* leaves in this study showed that they contain secondary metabolites like alkaloids, flavonoids, tannins, saponins etc as presented in (Tables 1).

Table 1: Phytochemical screening of *Gongronema latifolium* leaves

Phytochemicals	<i>Gongronema latifolium</i> leaves
Flavonoids	Positive
Saponins	Positive
Alkaloids	Positive
Tannins	Positive
Terpenoids	Positive
Steroids	Positive
Reducing sugars	Negative

The result of mineral analysis shown in table 2 reveals *Gongronema latifolium* leaves to be higher in calcium (333.40mg/100g) and least in chromium (0.25mg/100g). Other minerals present includes magnesium (38.90mg/100g), potassium (83.69mg/100g), sodium (31.02mg/100g), phosphate (51.13mg/100g), iron (4.88mg/100g), zinc (2.19mg/100g) and chromium (0.98mg/100g).

Table 2: Mineral composition of *Gongronema latifolium* leaves (mg/100g)

Minerals	<i>Gongronema latifolium</i> leaves (mg/100g)
Calcium	333.40 \pm 2.01
Magnesium	38.90 \pm 1.22
Potassium	83.69 \pm 1.30
Sodium	31.02 \pm 1.08
Phosphate	51.13 \pm 1.17
Iron	4.88 \pm 0.46
Zinc	2.19 \pm 0.09
Copper	0.98 \pm 0.04
Chromium	0.25 \pm 0.02

Values are means \pm SD for 2 determinations

The scavenging activities of DPPH radical and reducing power ability exerted by *Gongronema latifolium* leaves is shown in Fig.1 and 2. The scavenging effect of plant leaf in the range of 0.2-1.0mg/ml increased in a concentration-dependent manner.

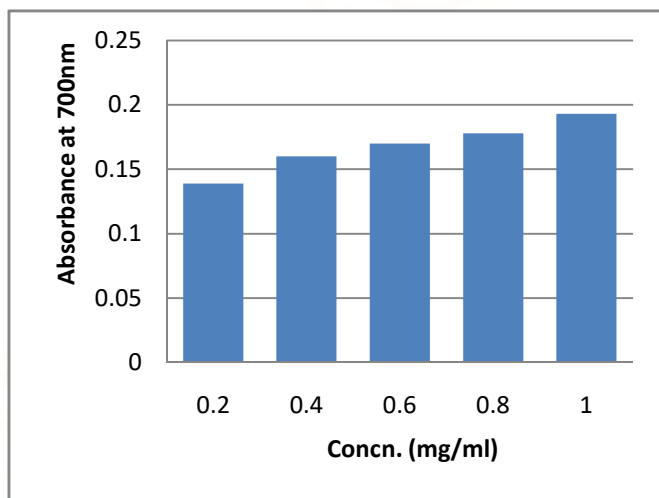


Figure 1: Reducing power ability of *Gongronema latifolium* leaves

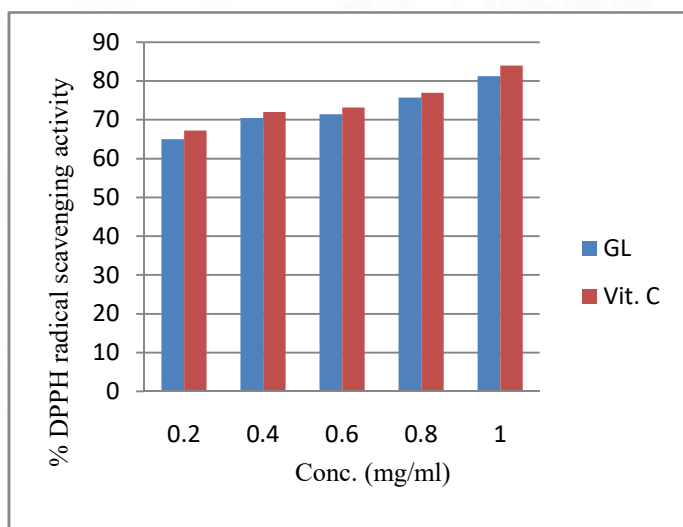


Figure 2: DPPH radical scavenging activity of *Gongronema latifolium* (GL) leaves

DISCUSSION

The presence of phyto-nutrients such as flavonoids, saponins, alkaloids and tannins in *Gongronema latifolium* leaves is an indication that the plant possesses the ability to scavenge for free radicals, thus health promoting in action¹⁵. The phytochemicals present in this study is similar to previously published phytochemicals in other plant leaves [16-19].

Calcium content of *Gongronema latifolium* (333.40mg/100g) is high compared to 295mg/100g of *Celosia argentea* [20], 1118.30mg/100g of *Annona muricata* and 1264.18mg/100g of *Vernonia amygdalina* [11-12]. Calcium, an important intracellular messenger, and cofactor for enzymes [21] plays roles in release of neurotransmitters and signaling. Zinc content of *Gongronema latifolium* (2.19mg/100g) is low compared to 5.42mg/100g of *Celosia argentea* [20] but high compared to 0.83mg/100g of *Annona muricata* and 1.42mg/100g of *Vernonia amygdalina* [11-12]. Sodium content of *Gongronema latifolium* (31.02mg/100mg) is low compared to 48.31mg/100g of *Vernonia amygdalina*, 69.49mg/100g of *Annona muricata* [11-12] and 71.32mg/100g of *Celosia argentea* [20]. Potassium content of *Gongronema latifolium* (83.69mg/100g) is low compared to 128.33mg/100g of *Celosia argentea*²⁰ but high when compared to 36.31mg/100g of *Annona muricata* and 62.79mg/100g of *Vernonia amygdalina* [11-12]. Copper content of *Gongronema latifolium* (0.98mg/100mg) is low compared to 1.95mg/100g of *Vernonia amygdalina* and 1.42mg/100g of *Annona muricata* [11-12] and 2.18mg/100g of *Celosia argentea* [20]. Iron content of *Gongronema latifolium* (4.88mg/100g) is low compared to 13.90mg/100g of *Annona muricata*, 32.20mg/100g of *Vernonia amygdalina* and 35.16mg/100g of *Celosia argentea* [20].

The reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron which is an important mechanism of phenolic antioxidant action [22]. Many reports have revealed that there is a direct correlation between antioxidant activities and reducing power of certain plant extracts [23-24]. The ability of *Gongronema latifolium* leaves to exhibit reducing power potential, shows its effectiveness to halt the oxidation of cellular macromolecules by oxidizing molecules that could arise from drugs or toxins metabolism.

The result of DPPH scavenging activity assay in this study indicates that *Gongronema latifolium* leaves is

potently active, suggesting that the plant contain agents that are capable of donating hydrogen to a free radical in order to remove odd electron which is responsible for radical's reactivity. The concentration-dependent scavenging activity of DPPH radical by *Gongronema latifolium* was found to be appreciable; an implication that the plant may be useful for treating radical related pathological damage.

In conclusion, the presence of phytochemicals, minerals and free radical scavenging potentials makes *Gongronema latifolium* leaves a beneficial medicinal plant. The phytochemicals present such as tannin and flavonoid supports the usefulness of *Gongronema latifolium* in the treatment of various infections

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