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Qualitative and Quantitative Phytochemical Analysis of *Gongronema latifolium* Leaf Extract

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Abstract

Phytochemical compounds are secondary metabolites produced by plants in which some have medicinal uses. The aim of this study is to qualitatively and quantitatively analyze the phytochemical composition of *G. latifolium* leaf extract. Fresh and healthy leaves of the medicinal plant of *G. latifolium* leaf were harvested, washed thoroughly in distilled water and dried at room temperature for 7 days. The dried leaf was milled to fine powder. The pulverized plant was weighed and used for the phytochemical analysis. Based on qualitative analysis, the results show the presence of tannins, alkaloids, saponins, flavonoids, terpenoids, reducing sugar, cardiac glycoside, protein and carbohydrate, while steroids was absent. The quantitative analysis of alkaloids, saponins, flavonoids and tannins were done and the results obtained were expressed as a mean± SEM in each group, analysed using ANOVA followed by Dunnett's test $P<0.05$ considered as significant. The quantitative analysis revealed that alkaloids, saponins, flavonoids and tannins had mean results of 9.85±1.50, 9.66±0.77, 6.83±6.25 and 4.54±6.70 mg/100mg respectively. This result shows that *G. latifolium* leaf extract possesses an appreciable level of phytochemicals which contributed to its efficacy in the treatment of diseases such as helminthiasis.

Keywords: *Qualitative, Quantitative Phytochemical, Analysis, Gongronema latifolium, Leaf Extract.*

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Introduction

Gongronema latifolium (Amaranth globe) is a tropical rainforest plant which belongs to the family Asclepiadaceae and genus *Gongronema* [1]. It is commonly grown in West Africa and is locally called "Utasi" by the Ibibios, Quas and Efiks; "Utazi" by the Igbos in South East and "Arokeke" by the Yorubas in South Western part of Nigeria. In Ghana and Senegal, the plant is referred to as "Akan-Asante aborode" and "Sever gasule" respectively [3]. It is an edible plant with green leaf, yellow flower and stem that produces milky latex when cut. It has a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh. *Gongronema latifolium* is widely believed to have strong nutritional and medicinal values. The leaf is rich in fats, proteins, vitamins, minerals and essential amino acids [4]. It is commonly used in soup as vegetable, or dried and applied as powdery spice. It is also consumed fresh and can be used in salad preparations [5] [6] [7]. In Sierra Leone, the root and stem are used as chewing stick or liquor. The liquor is obtained by boiling the sliced plant with lime juice or infused in water for over 3 days. It is then taken as a purgative for colic and stomach pains as well as to treat symptoms of worm infection [8] [9]. Apart from its nutritional values, *G. latifolium* is believed to possess strong medicinal qualities due to its composition of different active chemicals.

Some of the medicinal values of *G. latifolium* have also been scientifically validated [10]. The present review is focused on the detailed phytochemical, nutritional, ethno-medicinal and pharmacological updates of this plant. *G. latifolium* is a climbing perennial shrub up to 5m long. It climbs by twining around a support and can also run along the ground producing adventitious roots. The stem is hollow, soft and hairy with woody base and contains the latex. The leaves are green, simple, opposite and occasionally whorled, usually without stipules and the margins are nearly always entire; Petiole is about 3cm long; blade is broadly ovate to almost circular with deeply cordate base, acuminate apex and 3-veined basis [11]. The aim of this study is to analyze the qualitative and quantitative phytochemical in *G. latifolium* leaf extract.

Materials and methods

Collection of materials

Fresh and healthy leaves of *Gongronema latifolium* were harvested during rainy season from the plant naturally grown in Umuojogwo village Umuchu, Aguata local government area of Anambra State, Nigeria. The samples were taken to the department of Pharmacognosy and traditional medicine, Nnamdi Azikiwe University Awka, Agulu campus, Anambra state Nigeria for identification and extraction. The leaves of *Gongronema latifolium* were properly washed in distilled water and a known quantity (400g) were dried at room temperature for 7 days.

Method of extraction

Four hundred grams (400g) of the pulverized plant sample was macerated in 1000ml (1litre) of methanol over a period of 48 hours with intermittent shaking. The mixture was sieved using muslin cloth. It was further filtered using No.1 Whatman filter paper. The filtrate was concentrated using rotary evaporator at reduced temperature and pressure. It was further concentrated using water bath at 50^{o}C. The crude methanolic extract (CME) was stored in the refrigerator for further used.

Qualitative Phytochemical Analysis of *Gongronema latifolium* leaf extract

Test for alkaloids

The test was carried out according to the method of [12]. In this test, 0.5g of each plant extract was shaken with 5ml of 1% HCl and heated gently in a steam bath for 1 minute. Then 0.5ml of Wagner's reagent was added to each mixture (1ml) and observation made, a brick red colouration which indicated a positive result.

Test for saponins (frosting test)

This was carried out according to the method of [12]. Each plant of 0.3g extract was dissolved in 3ml of 95% ethanol and 2.0ml of each added into test tubes and shaken vigorously. They were then allowed to stand on the bench for 1 minute and observation made for the formation of stable frosts that indicated positive results.

Test for reducing sugar

Feling's test: Equal amount of fehling A and B reagent were mixed and 2ml of it was added to the plant extract and then gently heated the sample. Appearance of brick red precipitate indicated the presence of reducing sugars [13].

Test for flavonoids

A quantity (0.3g) of each extract of was dissolved in 3ml of 95% ethanol and heated. A small magnesium metal (0.1g) was added to the mixture followed by the addition of 3 drops of concentrated HCl. The occurrence of orange coloration was indicative of the presence of flavonoids compounds [14].

Test for tannins

Each extract (1g) was dissolved in 20ml of distilled water and filtered. Three drops of 10% of $FeCl_3$ were added to 2ml of the filtrate. The appearance of blackish-blue or blackish-green colouration was indicative of tannins some 2ml of the filtrate was added, 1ml of bromine water and a precipitate was taken as positive for tannins [12].

Test for carbohydrates

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish-brown precipitate formed which indicated the presence of carbohydrate [13].

Test for proteins

Protein was extracted from pulverized sample according the method described by [13].

Test for terpenoids

Standard processes were followed according to [12].

Test for cardiac glycosides (killer-killani test)

Ten milliliter (10ml) of each extracts (separately) were treated with 4ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layered with 1ml of concentrated sulphuric acid. A violet ring appears below the brown ring. While in the acetic acid layer, a greenish ring form just gradually throughout thin layer [13].

Test for steroids

Each plant extract of 0.5g was mixed with 2ml of acetic anhydride followed by 2ml of sulphuric acid. There is no colour change observed indicated the absence of steroids [13].

Quantitative phytochemical analysis of *Gongronema latifolium* leaf extract

The quantitative of the phytochemical present were determined using the methods [2] as shown below;

Alkaloid determination

Six grams (6g) of the material was weighed into a 500ml beaker, which was filled with 25% acetic acid in ethanol and left to stand for 4 hours. This was filtered and the extract was concentrated to one quarter of its original volume using a water bath. Drop by drop, concentrated ammonium hydroxide was added to the extract until it was completely precipitated. The entire solution was allowed to settle, after which the precipitate was filtered, dried and weighed.

Saponin determination

In 250ml of 25% ethanol, 25kg of fine powered leaves were poured. At 60^{o}C, the suspension was heated for 4 hours in a hot water bath with constant stirring. The residue was re-

extracted with another 200ml of 20% ethanol after the mixture was filtered. Over a water bath at roughly 90^{O}C, the mixed extracts were reduced to 40ml. the concentrate was placed to a 250ml separator funnel, which was then filled with 20ml of diethyl ether and violently agitated. The aqueous position was kept, but the other layer was thrown away. The purifying procedure was carried out three times. 60ml of n-butanol extract was washed twice with 10ml of 5% sodium chloride aqueous solution. In a water bath, the residual solution was heated. The sample was dried in the oven to a consistent weight after evaporation. Saponin content was determined as a percentage.

Determination of flavonoids

At room temperature, 10g of the fine powdered leaves were regularly extracted with 100ml of 80 percent aqueous methanol. The mixture was then filtered via a filter paper into a 250ml beaker that had been pre-weighed. The filtrate was placed in a water bath and allowed to dry completely before being weighed. The percentage flavonoid was calculated by subtracting the percentage flavonoid from the total flavonoid.

Determination of tannin

The folin-ciocalted reagent method was used to determine the total quantity of tannins. To 0.5ml of plant extract, 2ml of 2% Na_2CO_3 was added. After that, the mixture was left to sit at room temperature for 30 minutes. Ciollic acid (1mg/ml) was utilized as a standard. The sample's absorbance was measured at 765nm. All tests were performed three times, and the findings were calculated using a standard curve and represented as gallic acid equivalent (mg/g of extracted substance).

Results

The result of the qualitative phytochemical analysis of *Gongronema latifolium* leaf extract are shown in the table 1 below. *G. latifolium* has the following nine (9) phytochemical properties: Tannins, Alkaloids, Saponins, Flavonoids, Terpenoids Cardiac glycosides, proteins, reducing sugar and carbohydrates. Steroids are absent.

Table 1: Qualitative Phytochemical Analysis of *Gongronema latifolium* leaf extract

S/N	Phytochemicals	Concentration	Colour
1	Tannins	+	Blackish-blue
2	Alkaloids	++	Brick Red
3	Saponins	+	Frosth
4	Flavonoids	+	Orange
5	Terpernoids	++	Redish-brown
6	Reducing sugar	+	Brick-red
7	Steroids	-	-
8	Cardiac glycoside	+	Violet ring
9	Protein	+	Ring
10	Carbohydrate	+	Redish-brown

KEY: + = Trace/ mildly present**;** ++ Moderately present**;** Absence of substance

Table 2 shows the quantitative phytochemical analysis of Alkaloids, Saponins, Flavonoids and Tannins. Alkaloids, saponins, flavonoids, and tannins measures 9.85±1.50, 9.66±0.77, 6.83±6.25, and 4.54±6.70.

Table 2: Quantitative Phytochemical Analysis of *Gongronema latifolium* (mg/100mg)

G. latifolium	Alkaloids	Saponins	Flavonoids	Tannins
Replication 1	9.85±1.50	9.66±0.77	6.83±6.25	4.54±6.70
Replication 2	9.85±1.50	9.66±0.77	6.83±6.25	4.54±6.70
Replication 3	9.85±1.50	9.66±0.77	6.83±6.25	4.54±6.70

Discussion

Qualitative and quantitative phytochemical analysis of *G. latifolium* leaf extract shows the presence of various phytochemicals: alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, proteins, reducing sugar and carbohydrates were found present while steroids are found absent. The presence of these phytochemicals' constituents gives more authenticated to the findings of [15] where alkaloids, flavonoids, tannins, and saponins were detected in *G. latifolium* leaf extract. Moreso, several studies have proven that these metabolites have varied pharmacological efficacy in man and animals. The phytochemicals are naturally occurring chemical in plants which serves as medicine for the protection of human disease; the phytochemical are also nonnutritive plants chemical that has disease preventive properties [16] [17] [18]. Alkaloids are naturally occurring chemical compounds that contain mostly basic nitrogen atoms. Alkaloids have a wide range of pharmacological activities including antimalarial, antiasthma, anticancer, anti-helminthiasis, etc. Alkaloids have found use in traditional or modern medicine, or as starting materials for drug discovery [19] [18]. Some plants that possess alkaloids are known for decreasing blood pressure and balancing the nervous system in case of mental illness [18]. Flavonoids are known to inhibit lipid-peroxidation, platelet aggregation, capillary permeability and fragility, cyto-oxygenase and lipoxygenase enzyme activities. They manifest these effects as antioxidants, free radicals scavengers and chelators of divalent cation [20]. Presence of flavonoids might be responsible for its use as anti-inflammatory effects on both acute and chronic inflammation [21].

Conclusion

G. latifolium leaf extract has a good number of phytochemical constituents that qualifies it as a medicinal plant used in the treatment of human ascariasis [22]. This ascertains its efficacy in treating infectious diseases. Also, the presence of most general phytochemicals especially alkaloids with highest content may possibly be used in folk medicine for the treatment and prevention of some disease such as ascariasis, strongyliosis etc [22].

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