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Research Article

CYTOTOXICITY AND ANTIOXIDANT SCREENING OF SOME SELECTED NIGERIAN MEDICINAL PLANTS

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ABSTRACT
Nigerian medicinal plants (*Gongronema latifolia, Besella alba linn,* and *Telfairia occidentalis*) were screened for cytotoxicity and anti oxidant activities using standard methods. The results revealed that all the selected plants were relatively non-toxic. This bioactivity study using brine-shrimps gave LC_{50} = 1175.16 µg/ml for the most inhibitory activity in *Gongronema latifolia* leaf .The extracts possessed very low cytotoxicty to brine shrimps when compared to reference standard potassium dichromate LC_{50}=180.142 µg/ml). *G. latifolia* contained the highest amount of bioactive constituents. The importance or relevance of the phytochemical constituents is discussed with respect to the role of these plants in ethnomedicine in Nigeria. Rapid radical scavenging screening using thin layer chromatographic plate (TLC) indicated that G. latifolia had the highest antioxidant capacity. The high amount of the photochemical (polyphenol) is attributable to more potent radical scavenging effects as shown by *G. latifolia* stem and leaf extracts. From these results we infer that the plants are relatively safe for the purposes utilized.

KEYWORDS Cytotoxicity, ethnomedicine, phytochemicals, *Telfairia occidentals, Gongronema latifolia,Besella alba linn.*

INTRODUCTION

In Nigeria, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. Such medicinal plants include *Gongronema latifolia (Fam: Asclepiadaceae), Telfairia occidentalis (cucurbitaceae), and Besella alba linn (Besellaceae). Gongronema latifolia*, a non-woody forest product is a leafy vegetable climber found in South Eastern Nigeria and some other parts of sub-saharan Africa. It is highly nutritional, rich in minerals, vitamins and proteins; it has been used in the treatment of diverse diseases [1]. This plant has been very useful in the management of diabetes and a good supplement in women after child birth. [2].

Telfairia occidentalis is a vigorous perennial vine, growing to 10m or more in length. The stems have branching tendrils and the leaves are divided into 3– 5 leaflet. The fruits are pale green, 3 – 10 kg in weight, strongly ribbed at maturity and up to 25cm in diameter. The seeds are 3– 5cm in diameter [3]. The young succulent shoot and leaves are used as vegetables. It is rich in amino acids, vitamins and minerals. It is a ready source of proteins because of its ability to synthesize amino acids from a wide range of virtually available primary materials such as water, carbon dioxide and atmospheric nitrogen [4]. According to Oboh, (2005)[5] *Telfairia occidentalis* prevents against garlic – induced oxidative stress.

The plant also prevents the occurrence of abdominal pain, small intestine obstruction, dermatitis, asthma and increase of bleeding which are caused by garlic.

Besella alba linn belongs to the family Besellanceae. It is an annual herbaceous plant, a climber and can be grown on fences. According to Lin Nung, (1984), it is an anti-inflammatory agent and intestinal lubricant. It is contra indicated in pregnancy. In Thailand, the fresh leaves are used topically for ring worm. It is also antidandruff. Other medicinal uses of the plant include its use in the treatment of dysentery, joint pain, constipation, traumatic injury etc [6].

These selected plants because of the numerous oral claims on their efficacy in the management of diverse ailments and nutritional potentials, are used heavily in one way or the other in Nigeria. The present study was designed to evaluate these plants for some phytochemicals and as well screen same for antioxidant activity and cytotoxicity using brine shrimps.

MATERIALS AND METHODS

Plant Materials

The leaves and stems of the selected medicinal herbs, *Gongronema latifolia*, *Besella alba linn* and *Telfairia occidntalis* were purchased from Anyigba central market, Kogi State, Nigeria. The plant materials were washed with water to remove dirts and were air dried in the laboratory for two weeks. The dried plant materials were pulverized using pestle and mortar.

Preparation of crude methanolic extracts

Cold extraction method was employed. 20g of the powdered samples were weighed into conical flasks. 150ml of pure methanol was added and left for 72 hours. The mixtures were filtered and the filtrates were concentrated using rotary evaporator.

Chemicals

Methanol, Folin-Ciocalteu reagent, potassium dichromate, ferric chloride, and amyl alcohol were products of BDH. DPPH (2, 2-diphenyl – 1 – picrylhydrazyl) was purchased from Sigma Chemical Company (Germany).
Tannic acid used was M & B product, All other chemicals and reagents used were of analytical grade.

Total phenol, tannin and flavonoid determination.

The total phenol composition was determined using the folin-ciocalteu reagent as described by McDonald et al (2001) [7]. The method of Harbone, (1973) was followed in the determination of the total flavonoids content. The colorimetric method of Van-Burden and Robinson (1981) was employed in the determination of tannin content [8,9].

Cytotoxicity to brine – shrimps

Modified methods of Solis et al (1992) [10] and Potduang et al, (2007) [11] were used to determine the inhibitory activity on Artemia Salina. 50µl of different concentrations of crude methanolic extracts (1000, 500, 250 and 125 µg/ml) and control (methanol) was added into graduated vial bottles containing 10 newly hatched brine – shrimps in 5ml of artificial sea water, then incubated at room temperature for 24 hours. All samples were repeated in two wells to make the overall tested organisms of twenty for each. The living brine-shrimps were counted under a hand-magnifying lens. Same procedure was followed using potassium dichromate as the reference standard or positive control and data analysed based on U.S., E.P.A. Probit analysis programme version 1.5 to determine the LC_{50} at 95% confidence limit [12].

Rapid radical scavenging screening.

The methods of Mensor et al, (2001) [13]; Burits and Bucar [14] (2000); and Adebayo et al [15], 2007 was followed in the screening for the antioxidant property of the extracts. With the aid of capillary tube, stock solution (1mg/ml) of extracts were spotted on silicia gel thin layer chromatographic (TLC) plate and developed with a solvent system of ethanol: methanol (90:10). After development, the chromatograms were dried and sprayed with a 0.3mM solution of the stable radical DPPH. Yellow spot formed against purple background were taken as positive results. The duration for the development of yellow colour indicated whether the antioxidant activity was strong or not.

Statistical Analysis

Data are reported as the mean ± S.E. of three determinations. LC_{50} values for all the experiments were computed using the United States, Environmental Protection Agency Probit analysis programme version 1.5 [15].

RESULTS AND DISCUSSION

The result of the cytotoxicity screening is as presented on table 1. All extracts showed mild brine-shrimp inhibition, when compared with the reference standard used with LC_{50}=180.14µg/ml. The higher the LC_{50} value the lower the toxicity. *Gongronema latifolia* leaf extract exhibited the highest toxicity on the organism with 53.75% lethality, but this is not comparable with the reference standard with 80% lethality. Generally all the plants showed mild cytotoxicity when compared with the positive control. The resident phytochemicals could be contributory to the relatively non-toxic property exhibited by the plants. Table 2 summarizes the quantitative determination of some phytochemical constituents of the selected medicinal herbs. High quantity of flavonoids was found in all the plant samples. *Gongronema latifolia* stem recorded the highest flavonoid content (30.60±7.220%). Similarly the values of phenolic compounds and tannins were high in this plant compared to others. *Telfairia occidentalis* recorded the lowest content of the phytochemicals analysed.

The medicinal plants contained low level of tannins. Also *Gongronema latifolia* had the highest amount of tannins (3.563±0839%). Flavonoids are anti-inflammatory, anti-tumor, anti-viral,

Table 1-Result of the cytotoxicological effect of the crude methanolic extracts of the medicinal plants on brine-shrimps.

Medicinal plant	% Lethality	LC_{50} (µg/ml)
Gongronema latifolia (leaf)	53.75	1175.16
G. Latifolia (Stem)	47.50	2238.54
Besella alba linn (leaf)	48.75	1200.39
B. alba linn (stem)	32.50	1887.75
Talfairia Occidentalis (leaf)	33.00	1392.60
T. Occidentalis (stem)	31.21	1577.67
Potassium dichromate	80	180.14

Table 2-Phytochemical constituents of the leaf and stem extract of the selected medicinal plants.

Medicinal Plant	% Phenol	% Flavonoid	% Tannin
G. latifolia (leaf)	4.857±1.145	20.70±4.882	3.563±0.839
G. Latifolia (Stem)	4.857±145	30.60±7.220	3.002±0.708
Besella alba linn (leaf)	0.288±0.166	0.50±0.300	0.179±0.104
B. alba linn (stem)	0.289±0.200	17.50±0.120	0.117±0.067
Talfairia Occidentalis (leaf)	0.262±0.151	4.80±2.780	0.119±0.069
T. Occidentalis (stem)	0.274±0.158	0.70±0.470	0.129±0.075

Each value in the table was obtained by calculating the average of three determinations ± standard error of mean (S.E.M).

Table 3- Examples of therapeutic uses of the selected Nigerian medicinal plants.

Botanical name/Family	Local Nigerian names	Plant part tested	Claimed therapeutic use
Bessella alba linn (Besellaceae)	Ugbologi	Leaves and stem	Anti-inflammatory, antidandruff, constipatation, cure of joint pain, stop wound bleeding, used externally for eczemma, traumatic injury.
Telfairia occidentalis (Curubitaceae)	Ugwu	Leaves stems	Used as food medicine to prevent abdominal pain, asthma, dermatitits etc
Gongronema latifolia benth (Asclepiadaceae)	Utazi	Leaves and stems	Antimalaria herb, used to cure cough, high blood pressure, stomach aches. Used in the management of diabetes and associated symptoms.

Table 4- Radical scavenging abilities of the methanolic extracts from the selected medicinal plants using rapid DPPH, TLC screening

Plant species	Reaction speed	Intensity of spots
Gongronema latifolia (leaf)	Fast	+++
G. Latifolia (Stem)	Fast	+++
Telfairia occidentalis (leaf)	Very slow	+
T. occidentalis (stem)	-	-
Besella alba linn (leaf)	Very slow	+
B. alba linn (stem)	-	-

+++ Strong intensity of yellow colouration , ++Intermediate intensity of yellow colouration , + Weak intensity of yellow colouration , - No yellow colouration.

antiplateletes [16,17]. Flavonoid also are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity [18,19,20]. Flavonoids intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity. This may be the reason *Besella alba* Linn is used as anti-inflammatory agent and *Gongronema latifolia* as antimalarial, cough remedy, and in the management of diabetes and associated symptoms. Tannin have stringent properties , hasten the healing of wounds and inflamed, mucous membranes. These probably explain why *Besella alba linn* is used in treating wounds. Among the plants studied, *G. Latifolia* had the highest mount of phenols as seen on table 2. Phenols act as free radical chain reaction terminators thereby acting as antioxidant [21]. Phenols also have a potential of combating oxidative stress syndrome causative of some neurodegenerative diseases and cardiovascular diseases. These properties bestow high medicinal activities on the extracts of the plants studied. The result of the rapid radical scavenging screening is as presented on Table 4. *Gongronema latifolia* leaf and stem showed the highest antioxidant activity *T. Occidentalis* and *B. alba Linn* showed weak intensity of yellow colouration affirming weak antioxidant capacity.

The generation of the reactive oxygen species (ROS) beyond what the

ability of the body can cope with leads to oxidative stress [22,23]. Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases like: artherosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS [24]. The use of DPPH scavenging assays in assessing the cell membrane integrity/cell membrane stabilizing capacities of plant constituents has given explanations as to the possible ways by which phytomedicine could help to reduce diseases caused by infections, inflammation and oxygen radical generation affecting the cell membrane[25]. The model of scavenging DPPH free radicals used in the rapid screening method commonly employed for evaluating antioxidant activities based on their abilities to donate hydrogen ion [26]. The DPPH is a free radical stable at the room temperature. The methanolic solution gives a purple colouration which when reduced by an antioxidant molecule give rise to a yellow solution. The low polyphenolic content of *T. occidentalis* and *B. alba* linn extracts may account for their very weak antioxidant activities or inactivity.

CONCLUSION

The result of the present study showed that the extract of *G. latifolia* leaf and stem contain high amount of tannins, phenolic compounds, and flavonoids. It also exhibited high percentage lethality even though not comparable with the standard used. The selected plants screened showed mild cytotoxicity and could be well integrated into the Nigerian ethnomedical system and foodstuff as they are readily used.

The polyphenolic constituents of these plants could be contributory to their ethnomeidical use. The plants have high potentials as antioxidants, owing to high amount of flavonoids and phenolic compounds.

This study, therefore, has provided some biochemical basis for the ethnomeidical use of extracts from these selected Nigerian medicinal plants in the treatment and management of diverse ailments.

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