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Phytochemical and Cytotoxic Screening of Selected Medicinal Plants (*Byrsocarpus coccineus, Terminalia avicennioides* and *Anogeissus leiocarpus*) Using Brine Shrimp (*Artemia salina*) Lethality Assay

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Authors' contributions

This work was carried out in collaboration among all authors. Author CEU performed the laboratory work, analyzed the data and wrote the manuscript. Author OATE designed the experiments, critically reviewed and edited the manuscript. Author RAA reviewed and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Three medicinal plants, were investigated based on their ethno-medicinal uses. *Byrsocarpus* coccineus (*B.C*), *Terminalia avicennioides* (*T.A*) and *Anogeissus leiocarpus* (*A.L*) are used traditionally in the treatment of various ailments in Nigeria. Proximate and mineral analyses were carried out on the leaf, stem and root of the three plants. Phytochemical composition and antioxidant activities of the aqueous, ethanol and pet ether (leaf, stem and root) extracts, of the three plants were determined and the extracts were subjected to cytotoxic screening using the in vivo brine shrimp lethality tests. The proximate and mineral analyses show appreciable dietary nutrients in the three plants. Phytochemical analyses of *B.C*, *T.A* and *A.L* (leaf, stem and root) extracts, showed the presence of bioactive compounds, such as alkaloids, tannins, saponins, flavonoids, steroid and phenol. Antioxidant activities (2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric

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oxide, lipid peroxidation and reducing power), increase in all the plant extracts in a dose dependent manner. The results of brine shrimp lethality tests indicate that plant extracts of *B.C*, *T.A* and *A.L* except *A.L* stem aqueous extract (130.72 µg/ml), *T.A* leaf aqueous (130.15 µg/ml) and root aqueous extracts were moderately cytotoxic, while the others were highly cytotoxic. *B. coccineus* leaf ethanol extract (17.31 µg/ml) was the most cytotoxic. The result shows that *B. coccineus* leaf ethanol extract has significant antioxidant activity and is cytotoxic to brine shrimp even at low concentration giving credence to its ethno-medicinal uses.

Keywords: Byrsocarpus coccineus; Terminalia avicennioides; Anogeissus leiocarpus; phytochemicals; antioxidants; brime shrimp lethality.

1. INTRODUCTION

From antiquity, different novel drugs and new chemical entities (NCE) have been developed from a variety of medicinal plants, through pharmacognostical and pharmacological research. In developing countries, medicinal plants and their products are very important in primary health care, and are a growing source of alternate medicine [1]. The health benefits of medicinal plants are associated with their antioxidant activities and biological effects on cellular processes [2]. There is need for more research to determine the cytotoxic effects of medicinal plants used to treat a variety of ailments. Therefore, we decided to study cytotoxicity of three medicinal plants; *Byrsocarpus coccineus* (*B.C*), *Anogeissus leiocarpus* (*A.L*) and *Terminalia avicennioides* (*T.A*), frequently used by herbal practitioners for the treatment of a variety of ailments, in the south western region of Nigeria. *B.C* known as Amujewewe or Ado kantikanti (Yoruba vanacula name) is a scandent shrub widely employed as a medicinal remedy for various disease conditions in West Africa. Many biological activities such as antioxidant, anxiolytic, sedative [3], antiplasmodial [4] and antimicrobial properties [5], have been reported. *A.L* known as *Ayin* or Orin-odan (Yoruba vanacula name), is a deciduous tree, traditionally used to treat diarrhoea, dysentery, malaria and bacterial infections. This plant has been shown to have antimicrobial, antifungi and antiproliferation activities [6]. *T. A* known as *Idi* (Yoruba vanacula name) is a branched shrub, with a rough grey corky bark. T.A plant extracts have been shown to have antimalarial, hematological, and antioxidant activities [7].

Cytotoxicity study of these plants were carried out using the brine shrimp lethality assay, which is based on the ability to kill *Artemia nauplii* brine shrimp, cultured in the laboratory. This assay was proposed by Michael et al. [8] and developed later by Vanhaecke et al. [9] and Sleet and Brendel [10]. The brine shrimp lethality assay is a low cost preliminary assay used to investigate general cytotoxicity *in vivo*, which can then be backed by more specific and complex bioassays [11]. In this study cytotoxic screening of the aqueous, ethanol and petroleum ether extracts of the leaf, stem and root of *B.C, A.L* and *T.A,* were carried out using the brine shrimp lethality assay to determine the most cytotoxic extract.

2. MATERIALS AND METHODS

Plant samples (wild type) were collected from Southern part of Nigeria, and authenticated at the Department of Botany, University of Lagos, Lagos, with voucher numbers: *B.C* 7491, *A.L* 7475 and *T.A* 7474.

2.1 Preparation of Plant Extracts

The fresh leaves, stem bark and roots of the three plants were air dried for one week and ground into powdered form. 1kg of the plant sample was extracted by maceration with ethanol at 28 ± 2^0C, and concentrated to dryness using a rotary evaporator at reduced pressure [12]. Dried samples were stored at −20°C until further use.

2.2 Phytochemistry Analysis

Phytochemical constituents of the aqueous and ethanol extracts of the three plants were identified, using standard as described by Sofowora [13], Trease and Evans [14]. The presence of flavonoids, tannin, saponin, steroids, phenols, alkaloid (Mayer's reagent and Dragendroff's reagent), terpenoid (Salkowski test) and cardiac glycosides (Keller-Killani test) were tested for in the different plant extracts.

2.3 Proximate Analysis

Proximate analysis of *B.C, A.L* and *T.A,* leaf, stem and bark plant samples were carried out to

determine the carbohydrate, protein, crude fat, moisture content, ash content and crude fiber composition of the different plant samples by the method of Association of Official Analytical Chemists [15].

2.4 Mineral Analysis

Macro and micronutrients analyses were carried out on the leaf, stem and root of the three plant samples, using Perkin Elmer; Analyst 700, single beam atomic absorption spectrometer. Laboratory procedures for the preparation and determination of macro and micronutrients were used as outlined by Shah et al. [16] for plant samples. Macro and micronutrients were prepared and determined by Shah et al. [16] method for plant samples.

2.5 Antioxidant Screening

The following antioxidant activities were evaluated: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), Nitric oxide radical scavenging (NO), Lipid peroxidation and Reducing Power, according to standard procedures.

2.5.1 DPPH radical scavenging assay

The free radical scavenging effects of the plant extracts against DPPH were observed according to the method of Ursini et al [17]. Oneml of 0.1 mM solution of DPPH in ethanol was added to 1 ml of extract in water at different concentrations (20-100 µg/ml). The mixture was incubated at room temperature for 30 min in the dark, after shaken vigorously. The absorbance of the solution was measured at 517 nm against blank. The DPPH percentage inhibition was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_0-A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the blank and A_1 was the absorbance of the extract.

2.5.2 Nitric oxide scavenging assay

Nitric oxide scavenging assay was carried out by the method described by Govindarajan et al [18]. Sodium nitroprusside (5 mM in phosphate buffer saline) was added to different concentrations (20-100 µg/ml) of each extract. The mixture was allowed to incubate for 2 hr at 30°C, and 0.5 ml of Griess reagent (1% Sulfanilamide, 2% H_2PO_4 and 0.1% N-(1-naphthyl) ethylene diamine dihyrochloride) were added. Absorbance was taken at 550 nm, ascorbic acid was used as standard and methylated spirit as control

2.5.3 Lipid peroxidation assay

Lipid peroxidation assay was carried out by the method of Ohkawa [19]. The reaction mixture contained 1 mL of fowl egg yolk emulsified with phosphate buffer (pH 7.4) to obtain a final concentration of 25 g/l, plant extracts (20–100 µg/ml), and 100 µL of 1000 µM $FeCl_2$. The mixture was incubated at 37°C for 1 h. Lipid peroxidation was measured as MDA by using TBA–TCA reagent. 0.5 ml of freshly prepared 15% trichloroacetic acid (TCA) and 1.0 ml of 1% thiobarbituric acid (TBA) were mixed with the reaction mixture and heated in boiling water bath for 10 min. Once cooled to 28^0C temperature, the tubes were centrifuged at 3500 rpm for 10 min to remove the flocculent precipitate. The absorbance of the supernatant was measured at 532 nm spectrophotometrically to determine the MDA concentration. Ascorbic acid was used as standard.

2.5.4 Reducing power assay

The reducing power was determined according to the method described by Oyaizu [20]. Reaction was carried out in a mixture containing 1 ml of sample (20-100 µg/ml), 2.5 ml of 0.1 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1%, w/v potassium ferrocyanate [$K_3Fe(CN)_6$], by incubating at 50°C for 20 min. After addition of 2.5 ml trichloroacetic acid (10%, w/v), the mixture was centrifuged at 5000rpm for 10 min. The supernatant (5 ml) were s mixed with 0.5 ml of fresh FeCl3 (0.1%, w/v). The absorbance was measured against blank at 700 nm. Gallic acid was used as the control.

2.5.5 Brine shrimp lethality assay

Brine shrimp cytotoxicity assay was carried out to investigate the cytotoxicity and determine the lethal concentration (LC_{50}) value of the different plant extracts, using a method described by Krishnaraju [12]. Brine shrimps (*Artemia salina*) eggs were hatched in aerated filtered sea water, after 48 h. Ten shrimps nauplii were placed in each tubes containing 4.5 ml sea water, using a glass capillary. 0.5 ml of different concentrations (10, 100, 1000 µg/mL) of each extracts was added to each tube. The control consisted of 5 ml of seawater with 10 nauplii. The surviving (larvae) shrimps were counted after 24 h. All

experimental assays were done in triplicates. The LC_{50} was calculated using Probit analysis.

2.6 Statistical Analysis

Results were done in triplicates and expressed as mean ± SD. Data were analyzed by ANOVA. LC_{50} data were determined by the best fit line method and percentage mortality were calculated, using mean survival of treated and untreated brine shrimp larvae [12].

3. RESULTS AND DISCUSSION

In developing countries, medicinal plants play an important role in primary health care, this study was therefore carried out to screen three selected ethno-medicinal plants *B.C, A.L* and *T.A,* for interesting biological activities. Proximate analysis of *B.C, A.L* and *T.A* (leaf, stem and root) plant samples, showed the presence of carbohydrate, protein, crude fat, moisture, ash and crude fiber (Table 1). All plant samples have relatively high carbohydrate content compared to other components of the plants. *B.C* and *A.L* have relatively higher carbohydrate content than *T.A*, with *B.C* leaf having the highest carbohydrate content (65.18%) and *T.A* leaf the lowest **(**43.74%). *Croton tiglium* has been reported to have a carbohydrate yield as low as 15.51% [16]. In comparison, the plant samples are a good source of carbohydrate. All the plant samples had low protein content ranging from 6.305% (*B.C* root) to 12.75% (*T.A* root). This is an indication that all the plant samples do not contain sufficient protein needed humans, compared to legumes or nuts [21]. The crude fat content rage from 6.72% (*T.A* leaf) to 2.17% (*B.C* root). Dietary fat absorbs flavours and enhances food palatability. Food containing a fat caloric energy of about 2% is enough for humans, any excess intake of fat could lead to various ailments like cardiovascular disease [22]. The ash content was highest in *T.A* root at 4.36%, but *A.L* root had the lowest ash content (2.98%). High ash content is an indication of high mineral element in the plant [22].*T.A* leaf had the highest moisture content (9.78%), while *A.L* stem had the lowest moisture content (2.14%). The implication of low moisture content is that microbial growth and enzyme activities leading to spoilage will be hindered, thereby increasing shelve life of the leaf [23]. The highest crude fiber content was found in *T.A* stem (23.53%), while *B.C* had the lowest crude fiber content (12.75%). Dietary fiber enhances trace element absorption and improves bowel movement [24,25], it's also important in the management of ailments like gastrointestinal disorder [26]. Proximate analysis in this study revealed, that all the plant samples have appreciable dietary nutrients for the purpose of nourishing. Mineral analysis of the macronutrients showed a high concentrations of Sodium (Na), Potassium (K) and Magnesium in all plant samples except in *A.L* root (Table 2). A low concentration of Calcium (Ca) were observed in all plant samples but the in *T.A* leaf, the Ca concentration was high. Micronutrient analysis result showed the presence of Iron (Fe), Zinc (Zn), Copper (Cu) and Manganese (Mn) in all extracts. Lead (Pb) was found in *T.A* root and trace amount in *A.L* leaf but not in the other plant samples. The presence of the might be as a result of soil contamination. Nickel was found in trace amount only in *T.A* leaf. The chemical composition and mineral constituent of *B.C, A.L* and *T.A* were previously reported [27,28,29].

The phytochemical analysis of aqueous, ethanol and pet ether extracts of *B.C*, *T.A* and *A.L,* leaf, stem and root extracts showed the presence of bioactive compounds such as alkaloids, tannins, saponins, flavonoids, steroid and phenol in all the extracts. This result supports other [5,30,31]. The aqueous extracts were relatively high in saponins and flavonoids, and the ethanol extracts were relatively high in alkaloids, saponins and flavonoids, but phytochemicals were relatively low in the pet ether extracts (Table 1). These phytochemicals have been shown to have medicinal and physiological activities [13]. Phenolic compounds have been shown to possess biological activities like, antioxidant, antiproliferative and antiinflammation [32,33,34]. Alkaloids have been reported to exhibit pharmacological activities against some ailments such as cancer, hypertension and malaria [35]. Saponin have been shown to have biological properties like antioxidant and anticancer activities [34]. Flavonoid have been to exhibit pharmacological activities against cancer, free radicals and microorganism [36].

Free radicals have been implicated in many chronic diseases. Antioxidants ability to scavenge free radicals make them important in the management of such diseases. Natural antioxidant found in medicinal plants are preferred because of their relatively lower side effect compared to synthetic molecules [37]. The free radical scavenging effects of *B.C*, *A.L* and *T.A* were investigated by evaluating their antioxidant activity against free radical scavenging of DPPH and nitric oxide, lipid

Table 1. Proximate composition of selected medicinal plants

Plant extract	CHO %	Protein %	Crude fat %	Moisture %	Ash %	Crude fiber %
B.C leaf	65.18±3.01	7.32±0.42	3.23±0.12	8.01±0.13	3.56±0.01	12.75±3.42
B.C root	64.84±2.52	6.30±0.40	2.17±0.20	3.02±0.16	4.12±0.02	20.60±1.15
A.L leaf	49.46±3.13	10.20±0.80	5.83±0.24	4.62±0.22	3.26±0.01	18.63±2.10
A.L stem	58.31±3.02	6.36±0.72	3.89±0.51	2.14±0.31	3.82±0.04	20.12±1.01
A.L root	65.43±2.42	7.31±1.13	4.33±0.43	3.87±0.54	2.98±0.02	16.15±1.20
T.A leaf	43.74±2.01	10.52±1.34	6.72±0.31	9.78±0.41	3.13±0.02	19.16±1.17
T.A stem	48.95±1.62	9.61±1.01	2.37±0.32	6.55±0.23	4.03±0.01	23.53±3.01
T.A root	49.64±2.01	12.75±1.30	2.64±0.41	8.35±0.32	4.36±0.18	15.35±2.05

Key: CHO- Carbohydrate

- *Values are expressed as Mean ± SD*

Table 2. Mineral composition of selected medicinal plants (mg/100 g)

.

Plant extract	Na	K	Mg	Ca	Fe	Zn	Pb	Cu	Mn	Ni
B.C leaf	4.68±0.03	63.67	21.42±0.07	0.34±0.02	2.50±0.02	0.22±0.01	ND	0.046±0.005	0.70±0.07	ND
B.C root	4.39±0.02	29.19±0.03	21.67±0.05	1.31±0.07	0.12±0.01	0.07±0.01	ND	0.004±0.001	0.93±0.03	ND
A.L leaf	5.53±0.06	25.29±0.02	26.17±0.04	1.14±0.01	0.25±0.02	0.06±0.004	0.69±0.04	0.032±0.01	0.35±0.05	ND
A.L stem	3.34±0.06	20.73±0.04	27.39±0.03	1.99±0.01	1.55±0.02	0.07±0.005	ND	0.013±0.001	0.27±0.01	ND
A.L root	1.38±0.03	6.62±0.03	7.08±0.03	1.69±0.08	0.21±0.005	0.09±0.01	ND	0.013±0.001	0.40±0.01	ND
T.A leaf	10.74±0.17	84.54±0.01	41.09±0.02	32.36±0.03	1.21±0.001	0.18±0.01	ND	0.061±0/01	0.71±0.02	0.001
T.A stem	27.67±0.10	95.13±0.02	35.57±0.03	15.82±0.04	4.36±0.01	1.48±0.01	ND	0.878±0.02	1.01±0.01	ND
T.A root	20.56±0.07	42.55±0.05	41.82±0.07	1.17±0.04	2.50±0.02	0.98±0.01	12.32±0.05	0.122±0.01	3.63±0.03	ND

Key: ND - Not Determined

- *Values are expressed as Mean ± SD*

Table 3. Phytochemical composition of aqueous, ethanol and pet ether extract of selected plants (mg/100g)

Plant extract	Alkaloid (mg/100g)	Flavonoid (mg/100g)	Tannin (mg/100g)	Saponin (mg/100g)	C. glycoside (mg/100g)	Steriod (mg/100g)	Tapenoid (mg/100g)	Phenols (mg/100g)
B.C leaf (aqueous)	45.66±0.63	29.75±0.83	35.21±0.40	49.76±1.30	11.18±0.52	26.29±0.46	36.52±0.41	28.21±0.39
B.C leaf (ethanol)	43.27±0.09	40.33±0.09	40.20±0.06	55.12±0.20	19.61±0.08	26.85±0.08	32.58±0.04	31.84±0.08
B.C root (aqueous)	25.97±0.81	-	-	-	22.52±0.23	15.72±5.91	32.83±0.64	24.20±0.44
B.C root (ethanol)	40.88±0.09	28.93±0.05	12.86±0.09	16.49±0.07	30.19±0.12	36.04±0.08	37.62±0.16	22.76±0.12
B.C root (pet ether)	29.60±0.22	11.49±0.14	1.56±0.20	15.18±0.19	-	11.35±0.29	-	19.17±0.04
A.L leaf (aqueous)	20.31±0.69	17.76±0.32	14.57±0.84	62.83±3.25	18.35±2.78	26.66±0.17	27.99±0.52	18.81±0.33
A.L leaf (ethanol)	49.78±0.13	26.25±0.09	20.41±0.03	18.86±0.20	13.97±0.16	19.12±0.12	25.16±0.08	18.50±0.08
A.L leaf (pet ether)	30.31±0.04	5.91±0.46	5.63±0.12	23.46±0.06	11.60±0.16	6.02±0.21	10.33±0.17	16.75±0.04
A.L stem (aqueous)	29.34±0.56	-	14.88±0.72	53.29±1.02	18.06±0.41	29.61±0.41	33.24±0.52	29.90±0.22
A.L stem (ethanol)	50.13±0.04	19.21±0.05	23.82±0.03	43.17±0.07	11.48±0.12	27.68±0.16	23.89±0.12	19.67±0.08
A.L stem (pet ether)	30.67±0.14	3.36±0.36	6.79±0.09	28.98±0.20	10.66±0.12	8.23±0.29	30.09±0.24	16.79±0.07
A.L root (aqueous)	17.52±0.25	25.70±0.51	15.93±0.52	54.38±2.42	22.03±0.35	14.17±0.35	36.02±0.41	18.74±0.22
A.L root (ethanol)	44.07±0.09	17.08±0.09	25.61±0.11	36.07±0.20	8.05±0.04	17.81±0.12	24.92±0.16	18.62±0.04
A.L root (pet ether)	28.01±0.13	4.14±0.23	6.36±0.17	34.43±0.13	-	8.56±0.29	36.11±0.13	18.15±0.04
T.A leaf (aqueous)	27.70±1.63	29.79±0.26	25.55±0.56	-	20.48±0.35	30.02±0.64	15.45±0.64	27.28±0.39
T.A leaf (ethanol)	36.42±0.04	19.44±0.09	29.13±0.11	26.74±0.20	16.34±0.25	24.24±0.08	20.98±0.16	23.30±0.04
T.A leaf (pet ether)	23.23±0.31	3.77±0.04	10.03±0.26	17.42±0.06	-	3.77±0.25	36.85±0.20	16.79±0.11
T.A stem (aqueous)	27.48±0.44	-	11.39±0.44	66.89±0.56	19.57±0.23	24.49±0.46	33.20±0.12	14.64±0.50
T.A stem (ethanol)	27.30±0.75	13.85±0.05	19.68±0.31	29.50±0.20	15.77±0.16	29.48±0.08	20.08±0.16	29.27±0.16
T.A stem (pet ether)	23.76±0.13	11.86±0.14	15.22±0.17	24.51±0.20	11.15±0.12	8.93±0.25	31.15±0.08	19.99±0.07
T.A root (aqueous)	24.51±0.38	13.35±0.77	26.80±0.88	56.96±1.58	19.37±0.17	27.27±0.23	33.85±0.23	24.24±0.17
T.A root (ethanol)	34.47±0.04	10.54±0.09	30.10±0.06	35.55±0.20	-	25.55±0.08	-	16.59±0.12
T.A root (pet ether)	21.68±0.18	-	17.38±0.17	34.63±0.06	11.24±0.21	16.55±0.24	25.50±0.17	16.44±0.12

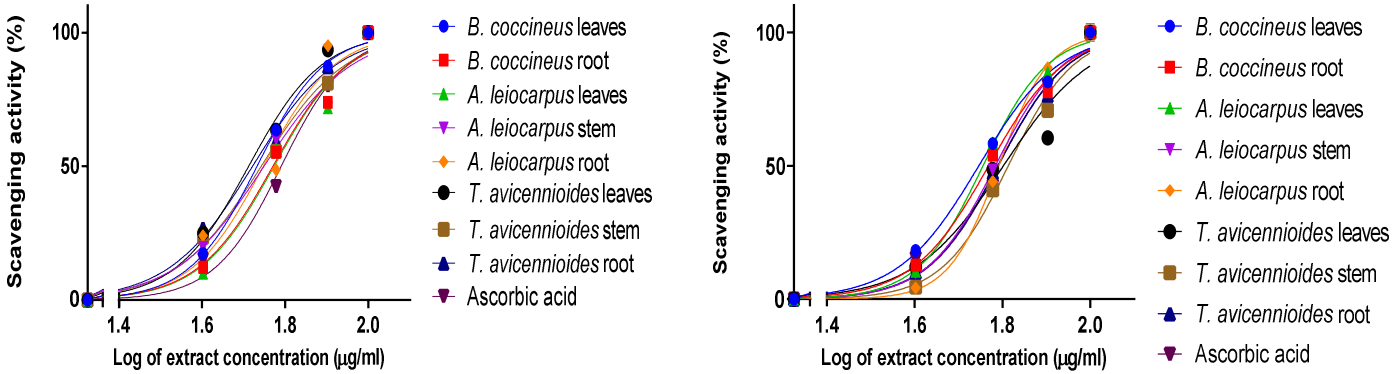

Fig. 1. DPPH scavenging activity (% inhibition) of aqueous extract (a) and ethanol extract (b) of selected plants

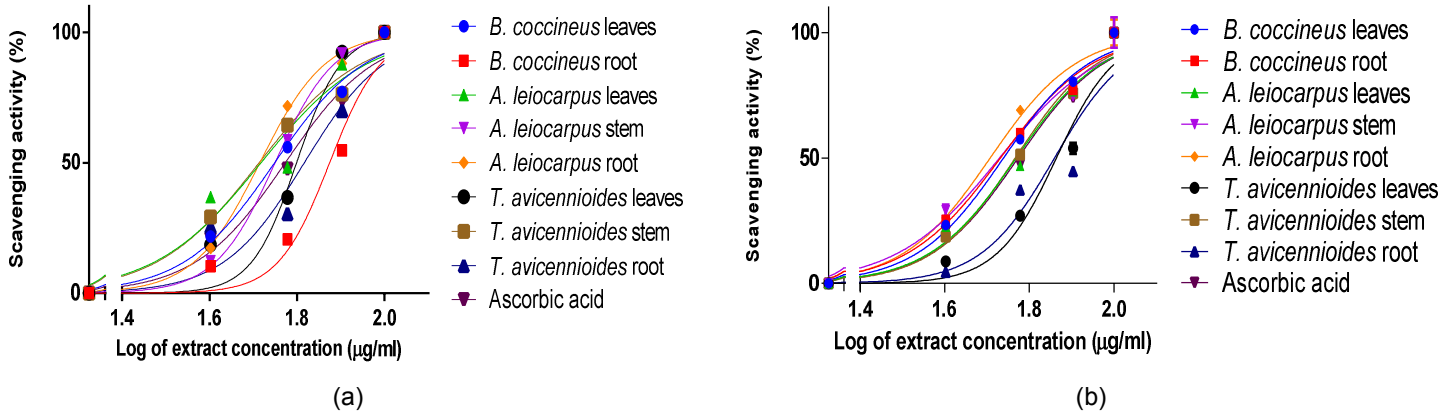

Fig. 2. Nitric oxide scavenging activity (% inhibition) of aqueous extract (a) and ethanol extract (b) of selected medicinal plants

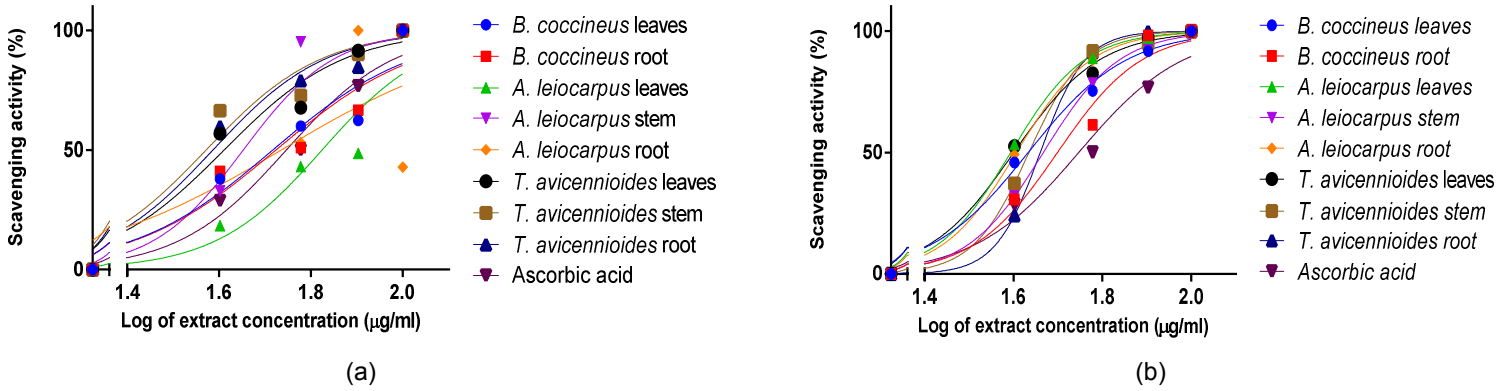

Fig. 3. Lipid peroxidation scavenging activity (%inhibition) of aqueous extract (a) and ethanol extract (b) of selected medicinal plants

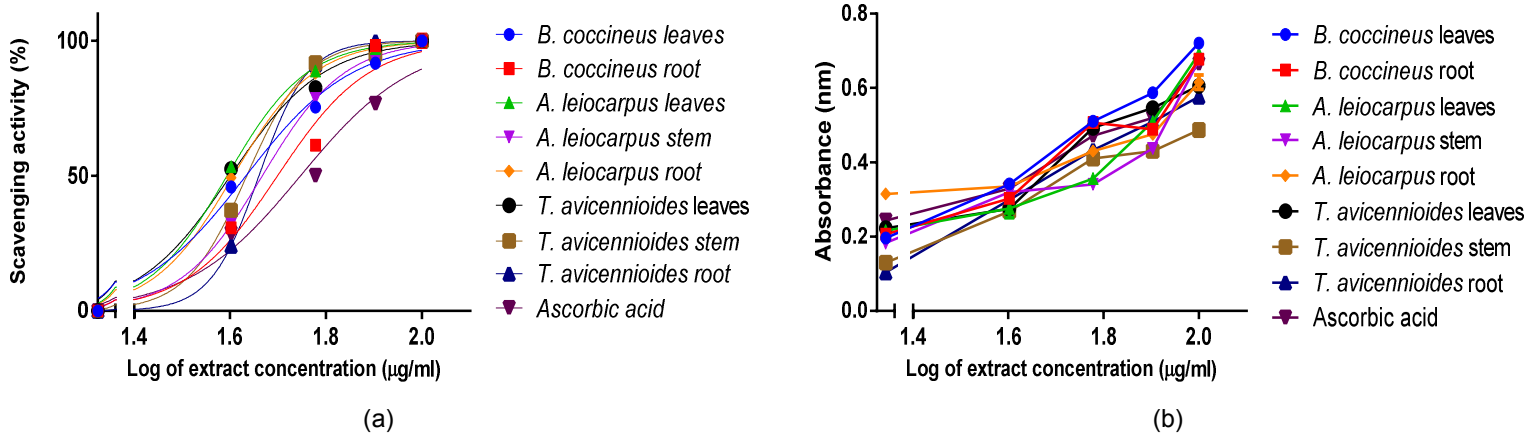

Fig. 4. Reducing power absorbance of aqueous extract (a) and ethanol extract (b) of selected medicinal plants

Table 4. Inhibition concentration (IC_{50}) (µg/ml) values for selected medicinal plant extracts

Plant extract	DPPH aqueous	DPPH ethanol	NO Aqueous	NO ethanol	Lipid aqueous	Lipid ethanol
B.C leaf	55.73±1.02	53.97±1.01	56.08±1.03	54.80±1.02	52.14±1.08	42.9±1.025
B.C root	58.92±1.03	58.68±1.02	64.91±1.03	53.87±1.03	52.97±1.08	49.98±1.04
A.L leaf	59.31±1.03	56.97±1.01	52.20±1.06	58.72±1.03	66.73±1.07	39.23±1.01
A.L stem	56.09±1.03	60.77±1.01	56.21±1.01	53.62±1.04	47.42±1.11	46.46±1.01
A.L root	55.87±1.04	56.71±1.04	51.88±1.01	50.96±1.03	52.03±1.11	40.42±1.01
T.A leaf	51.52±1.02	63.41±1.05	62.94±1.03	68.06±1.03	50.42±1.05	43.86±1.02
T.A stem	55.22±1.02	65.04±1.02	51.68±1.03	58.41±1.03	54.29±1.16	43.12±1.01
T.A root	52.63±1.02	61.53±1.02	65.55±1.05	62.32±1.06	50.62±1.05	45.72±1.00
Ascorbic acid	56.70±1.02	56.70±1.02	59.31±1.03	59.31±1.03	55.80±1.04	55.80±1.04

Key: DPPH- 2,2-diphenyl-1-picrylhydrazyl, NO- Nitric oxide

Table 5. Brine shrimp cytotoxicity data of selected plant medicinal extracts

S/N	Plant	Part extracted	Solvent used for extraction	Probit analysis LC50 (µg /ml)
1	*B.C*	Leaf	Aqueous	83.00
2	*B.C*	Leaf	Ethanol	17.31
3	*B.C*	Leaf	Petroleum Ether	28.51
4	*B.C*	Root	Aqueous	97.90
5	*B.C*	Root	Ethanol	19.16
6	*B.C*	Root	Petroleum Ether	39.00
7	*A.L*	Leaf	Aqueous	87.24
8	*A.L*	Leaf	Ethanol	22.90
9	*A.L*	Leaf	Petroleum Ether	37.15
10	*A.L*	Stem	Aqueous	130.72
11	*A.L*	Stem	Ethanol	21.24
12	*A.L*	Stem	Petroleum Ether	44.39
13	*A.L*	Root	Aqueous	83.00
14	*A.L*	Root	Ethanol	26.09
15	*A.L*	Root	Petroleum Ether	36.50
16	*T.A*	Leaf	Aqueous	130.15
17	*T.A*	Leaf	Ethanol	25.87
18	*T.A*	Leaf	Petroleum Ether	57.26
19	*T.A*	Stem	Aqueous	172.30
20	*T.A*	Stem	Ethanol	25.10
21	*T.A*	Stem	Petroleum Ether	55.95
22	*T.A*	Root	Aqueous	124.7
23	*T.A*	Root	Ethanol	25.19
24	*T.A*	Root	Petroleum Ether	62.4

Key: LC- Lethality concentration

peroxidation and reducing power activities. Different concentrations (20, 40, 60, 80, and 100 µg/ml) of the extracts were prepared. Extracts from *B.C*, *A.L* and *T.A* showed varying levels of inhibition of DPPH, nitric oxide, lipid peroxidation and reducing power activities, in a dose dependent manner, when compared to vitamin C s (Figs. 1-4). This finding is in agreement with the result of antioxidant activities of *B.C*, *A.L* and *T.A* previously reported [38-40]. The free radical scavenging (antioxidant) activities of *B.C, A.L* and *T.A* may due to the presence of phenols, flavonoid, saponin and alkaloids which are known to have antioxidant activity [41]. This probably contributed to their effectiveness as a medicinal plant.

Brine shrimp lethality is a preliminary and convenient assay for monitoring biological activities in plants, and the results in many instances correlates with cytotoxic and anti-tumour property [42]. The lower the LC_{50} value, the more cytotoxic the extract is [43]. Cytotoxicity of plant extracts were determined using the

Clarkson's toxicity index: non-toxic (LC_{50} >1000 µg/ml), low toxic (LC_{50} 500 - 1000 µg/ml), medium toxic (LC_{50} of 100 - 500 µg/ml) and highly toxic (LC_{50} of 0 - 100 µg/ml) [44]. After placing brine shrimp in test tubes containing sea water and different concentrations (10, 100, 1000 µg /mL) of each extract, results showed that all plant extracts of *B.C*, *T.A* and *A.L* were moderately cytotoxic, except *A.L* stem aqueous extract (LC_{50} 130.72 mg/ml), *T.A* leaf aqueous (LC_{50} 130.15 mg/ml) and root aqueous extracts (LC_{50} 124.7 mg/ml), while the other extracts with LC_{50} < 100 mg/ml were highly toxic. *B.C* leaf ethanol extract (LC_{50} 17.31 mg/ml) was the most cytotoxic extract (Table 5); this is in line with previous studies which shows that *B.C* reduced the viability of HT29 cells [45]. Studies have also shown that *B.C* also exhibited cytotoxic activities against human breast, ovarian and prostate carcinoma cell lines as been reported [46,47]. The significant lethality of *B.C* leaf ethanol extract to brine shrimp is an indication of the presence of potent cytotoxic components which will be further investigated.

4. CONCLUSION

Data obtained indicate that *B. coccineus* ethanol leaf extract was the most cytotoxic to brine shrimps when compared to *B.C* aqueous leaf extract, *B.C* pet ether leaf extract, *B.C* ethanol root extract, *B.C* aqueous root extract, *B.C* pet ether root extract, *A.L* aqueous leaf extract, *A.L* ethanol leaf extract, *A.L* pet ether, *A.L* aqueous stem extract, *A.L* ethanol stem extract, *A.L* pet ether stem extract and *T.A* leaf, stem and root extracts.

The high level of alkaloids, phenols and other phytochemicals and potent free radical scavenging (antioxidant) activities of *B. coccineus* ethanol leaf extract, probably contributes to the cytotoxicity of the plant extract against brine shrimps. Therefore, the present study suggests that *B. coccineus* leaf ethanol extract has significant antioxidant activity and is cytotoxic to brine shrimp, even at low concentration giving credence to its ethnomedicinal uses. Brine shrimp lethality assay was only able to assess the cytotoxic potential of the extracts, bioactive compounds in the plant extract should be further isolated and the mechanism of action determined.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Health Research Ethics Committee of the College of Medicine of the University of Lagos, Lagos, Nigeria. Approval number: CMUL/HREC/ 09/19/617.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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