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Antioxidant Activity and Phytochemistry Study of *Combretum obanense* Stem (Bak. F) Hutch. and Dalz

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Authors' contributions

This work was carried out in collaboration between all authors. Author MKT designed the study, performed the extraction and wrote the first draft author OWO performed the in vitro analysis and the statistical analysis. Author FIN collected and identified the plant. Authors CECU and MMI managed the analysis of the study and the literature searches. All the authors read and approved the final manuscript.

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ABSTRACT

Objective: The objective of the present study was designed to test the antioxidant activity and phytochemical property of the methanol/ methylene chloride (1:1) extract of *Combretum obanense*.
Place and Duration of Study: The International Centre for Ethnomedicine and Drug Development (InterCEDD), 110 Aku Road Nsukka, Enugu State, Nigeria, between January 2014 and July 2014.
Methodology: We had assessed methanol/methylene chloride (1:1) stem extract of *C. obanense*, for antioxidant potentials with the help of DPPH (1, 1-diphenyl-2-picryl hydrazyl) free radical scavenging, reducing power assay and phytochemical analysis.
Results: The extract was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 78.09, 80.13, 80.54, 80.45, and 76.99% at concentrations of 25, 50, 100, 200, and 400 µg/ml respectively while reducing power extract ranging from 0.7490±0.0017 to 0.16470.0007.

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Conclusion: The finding supports the traditional use of this plant in Nigeria traditional medicine. We recommend further research on these plant stem for possible isolation and characterization of the various active chemical substances which has the toxic and medicinal values.

Keywords: Free radical; antioxidant; DPPH assay; reducing power model.

1. INTRODUCTION

Combretum is a rainforest shrub, flower spikes up to 5 cm long. Leaves are glabrous and glandular beneath with pits in the axils of the main nerves, lamina elliptic, coriaceous, 8–12 cm long and about 4 cm broad. Main lateral nerves are prominently looped. It is widely distributed and native to tropical and southern Africa [1], about 5 species Madagascan, some 25 species to tropical Asiaand approximately 210 species tropical America. The genus is absent in Australia [2].

Several species of *Combretum* are used in African or Indian herbal medicine. The class of chemical compound known as Combretastatins were isolated from South African Bushwillow (*Combretum caffrum*), from which they get their name. One synthetic derivative, fosbretabulin disodum (combretastatin A4 phosphate) underwent preliminary study for the treatment of anaplastic thyroid cancer [3], but it was not effective enough to progress to more advanced trial. The species of *Combretum molle* is also recorded to contain antioxidants such as punicalagin, which is also found in pomegranates (*Punicagra natum*), a somewhat related plant. From three results of the antimicrobial screening of some species of *Combretum* such as *Combretum fragrans,* and *Combretum padoides,* the methanolic extracts of these species showed marked inhibition against Gram-positive bacteria, and were also good inhibitors of *Enterobacter aerogenes* [4]. *Combretum* extracts or isolates have shown *in vitro* bioactivities such as antibacterial, antifungal, anti-hyperglycemic, cytotoxicity against various human tumor cells lines, anti-inflammatory, anti-snake, anti-malaria, and anti-oxidant effect [5]. At least twenty four species of *Combretum* are well known in African traditional medicine, and used for the treatment of a variety of ailments and diseases, ranging from scorpion and snake bites, mental problems, heart and worm remedies to fever and microbial infection [6].

C. obanense (Bak. F.) Hutch. and Dalz. is a forest liana or scandent shrub with glabrous, coriaceous leaves and glandular beneath. It has elliptic lamina with main lateral nerves prominently looped, 8–12 cm long and 3.5–5.5 cm broad. The plant is found in rainforests of Southern Nigeria and is easily distinguished from other indigenous *Combretum* spp by the coriaceous leaves with looped lateral veins. However, there has not been any record of its ethnomedicinal uses [7].

The objective of the present study was designed to test the antioxidant activity of the methanol/ methylene chloride (1:1) extract of *C. obanensis*.

2. MATERIALS AND METHODS

2.1 Plant Material and Preparation of Extract

Combretum obanense stem bark was collected within the surrounding of Okutu in Nsukka Local Government Area of Enugu State, Nigeria in March 2013, Nigeria, and were identified and authenticated by Mr. Alfred Ozioko of International Centre for Ethnomedicine and Drug Development. The voucher specimen (INTERCEDD 022013) is deposited at the same center.

The air-dried and powdered plant material (5 Kg) was macerated in a mixture of CH_2Cl_2-MeOH (1:1) for 24 h. Removal of the solvent *in vacuo* in a rotary evaporator provided an organic extract (200 g).

2.2 Phytochemical Analysis of the Extract

The methanol: methylene Chloride (1:1) extract of *Combretum obanense* was subjected to the following phytochemical tests described by Trease and Evans [8].

2.3 *In vitro* Antioxidant Tests

2.3.1 Test on reducing power model

The reducing power was determined by the method of Athukorala [9]. In this model, 1.0 ml extract of different concentrations (25, 50, 100,

200 and 400 µg/ml) were each mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6). To each of the mixtures, 2.5 ml of potassium ferrocyanide (30 mM) was added and incubated at 50ºC for 20 min. Thereafter, 2.5 ml of 0.6 M trichloroacetic acid (TCA) was added to the reaction mixtures and centrifuged for 10 min at 3000 rpm. The upper layer of each solution (2.5 ml) was decanted and mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM). The change in color was observed and then the absorbance of each mixture was measured at 700 nm using a UV-Vis Spectrophotometer. The same treatment was used for the different concentrations of the standard, and one for the normal control. Ascorbic acid was used as the positive control [10], with distilled water as the normal control. A higher absorbance of the reaction mixture indicated greater reducing power.

2.3.2 Test for free radical scavenging activity: DPPH assay

DPPH radical scavenging activity was measured using the methods of (Cotelle) [11] and (McCune and Johns) [12] with some modifications. The reaction mixture (3.0 ml) was prepared and it consisted of 1.0 ml of DPPH in methanol (100 µM), 1.0 ml of methanol and 1.0 ml each for the different concentrations of the extract and standard. The reaction mixtures were incubated for 10 minutes in a dark room, and then their absorbance were measured at 517 nm using a UV-Vis Spectrophotometer. The positive control (standard) used was ascorbic acid (Blois) [13]. The same treatment was given to the normal control that consisted of 1.0 ml of the 100 µM DPPH solution in methanol + 2 ml of methanol, and the absorbance was determined. The percentage of inhibition was calculated using the formula:

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

Where:

A_0 is the absorbance of control
A_1 is the absorbance of test.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

Table 1 shows the phytochemical constituents of *C. obanense* stem.

3.2 The Reducing Power Ability of *C. obanense* Extract and Ascorbic Acid

Table 2 shows the results of the different absorbance values obtained from the reducing ability of *C. obanense* extract compared to that of the standard ascorbic acid at different concentrations.

3.3 The DPPH Free Radical Scavenging Assay

Table 3 indicates the absorbance values and percentage inhibition of different concentration of ascorbic acid and C. *obanense* using DPPH free radical scavenging assay. All values were significant at $p < 0.001$.

Table 1. Shows the phytochemical onstituents of *C. obanense* stem

S/N	Phytochemical	Presence
1	Alkaloids	+++
2	Carbohydrate	+++
3	Flavonoids	+++
4	Proteins	+++
5	Tannins	+++
6	Terpenoids	+++
7	Reducing sugar	+++
8	Glycosides	++
9	Oil	++
10	Resins	++
11	Saponins	++
12	Steroids	++
13	Acidic compounds	+
14	Anthraquinone	-

Key: +++ = high in concentration; ++ = medium in concentration;+ = low in concentration
- = absence; S/N= Serial Number

Antioxidant compounds act by several mechanism such as inhibition of generation and scavenging activity against reactive oxygen species (ROS); reducing power; metal chelation; activity as antioxidant enzymes; inhibition of oxidative enzymes, among others [14]. It has been established that oxidative damage caused by reactive oxygen species (ROS) leads to DNA lesions, loss of functions of enzymes, increased cell permeability, disturbed signaling over the cell and eventual necrotic cell death or apoptosis [14]. The aim of the present study was to evaluate the antioxidant potential of *C. obanense* using the DPPH scavenging assay and Reducing Power Assay Model.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule which is widely used to investigate radical scavenging activity. In DPPH radical scavenging assay, antioxidants react with DPPH (deep violet color) and convert it to yellow coloured α,α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the Radical-scavenging potential of the antioxidant [15,16].

The extract was capable of neutralizing the DPPH free radicals via hydrogen donating activity by 78.09, 80.13, 80.54, 80.45, and 86.99% at concentrations of 25, 50, 100, 200, and 400 µg/ml respectively. As shown in table 3, DPPH scavenging was increased in a concentration dependent manner compared to ascorbic acid, which was used as the positive antioxidant control in this investigation.

The reducing ability of a compound generally depends on the presence of reductants [17] which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom [18]. The presence of reductants in extract causes the reduction of the Fe3+/ferricynide complex to the ferrous form. Therefore, the Fe2+ can be monitored by measuring the formation of Perl's Purssian blue at 700 nm. Table 2 shows the reductive capabilities of the extract compared to ascorbic acid. Reducing power of the extract ranges from 0.7490±0.0017 to 0.1647±0.0007.

Table 2. The reducing power ability of *C. obanense* extract and ascorbic acid

Sample	Concentration (µg/ml)	Absorbance values±S.E.M.
Normal control	-	0.7220±0.0000
Ascorbic acid	25	0.8647±0.0007*
	50	1.6373±0.0084*
	100	1.0090±0.0006*
	200	1.1637±0.0015*
	400	1.4757±0.0033*
Combretum obanense extract	25	0.7490±0.0017*
	50	0.9430±0.0000*
	100	1.2037±0.0003*
	200	1.6007±0.0027*
	400	1.7003±0.0090*

** p < 0.01 (values compared to the control)*

Table 3. The percentage inhibition (%) of C*ombretum obanense* extract and ascorbic acid for the DPPH free radical scavenging assay

Sample	Conc. (µg/ml)	Abs. values ± S.E.M	Percentage inhibition (%)
Normal control	-	0.7180±0.0006	-
	25	0.1250±0.0000*	82.59
Ascorbic acid	50	0.0953±0.0003*	86.73
	100	0.0883±0.0003*	87.70
	200	0.0873±0.0003*	87.84
	400	0.0863±0.0009*	88.02
Combretum obanense extract	25	0.1573±0.0003*	78.09
	50	0.1427±0.0003*	80.13
	100	0.1393±0.0003*	80.54
	200	0.1403±0.0012*	80.45
	400	0.1647±0.0007*	86.99

**p < 0.01 compared to the control*

This observation could be attributed to the difference in the levels of the phytochemicals in the extract which are responsible for metal reduction probably due to their highly nucleophilic nature that enables them to readily donate proton to electron deficient centers to cause reduction.

4. CONCLUSION

From the result obtained from the experiment it is concluded that extract of *C. obanense* has good antioxidant property. The finding supports the traditional use of this plant and suggests the presence of biological active components which may be worth further investigation and elucidation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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