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Research Article

Antioxidant and Antiproliferative Activities of the Stem Bark Extract and Fractions of *Boswellia dalzielii* Hutch

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ABSTRACT

Boswellia dalzielii, the West African species of the frankincense tree, has several applications in ethnomedicine in the sub-region. The present work aims at evaluating the antioxidant and antiproliferative activities of *Boswellia dalzielii* stem bark. Antioxidant activity was evaluated through DPPH radical scavenging and ferric reducing antioxidant power (FRAP), which often correlates well with total phenolic and flavonoid contents. Antiproliferative activity was evaluated through growth inhibitory effects on *Sorghum bicolor* seeds. The dried, powdered stem bark of *B. dalzielii* was extracted with 70% methanol, yielding the crude extract that was partitioned successively into hexane and ethyl acetate; thereafter the aqueous mother liquor was also recovered. The ethyl acetate fraction, being most active in the bioassays, was subjected to Accelerated Gradient Chromatography (AGC) to give four sub-fractions (A-D). Phenolic content was found to be highest in sub-fraction C (481.20 ± 10.13 mg GAE/g) while flavonoid content was highest in methanolic extract (142.17 ± 4.82 mg RE/g). High antioxidant activities were recorded in the aqueous (IC_{50} 1.58) and methanol extracts (IC_{50} 1.99) using DPPH. FRAP assay gave high antioxidant capacity for aqueous extract (EC_{50} 1.00) and sub-fraction D (EC_{50} 1.25). In antiproliferative assay, sub-fractions C and D at 125 µg/ml gave the highest percentage inhibition (90%) followed by sub-fraction B (50%) at 250 µg/ml. These results show that the stem bark of *Boswellia dalzielii* has strong antioxidant and antiproliferative activities; justifying its application in ethnomedical practice and as a source of bioactive agents with potential to yield anticancer drug candidates.

Keywords: Antioxidant, Antiproliferative, *Boswellia dalzielii*, *Sorghum bicolor* seeds, Total phenolic content, Total flavonoid content

INTRODUCTION

Africans have maintained the age-old tradition of using plants as remedies for their health needs[1]. *Boswellia dalzielii* Hutch is a plant of the frankincense genus, *Boswellia* and family Burseraceae. It is a tree that is commonly found in Northern Nigeria and the West African savannah. It is often used among the local population to treat several disease conditions[2-5]. In Cameroon, people use the leaves of *B. dalzielii* to protect maize, millet and sorghum against weevil attacks[6]. The extract of the leaves is used for the treatment of diarrhoea and the gum resin of this plant is used locally for fumigation of clothes and houses[7]. The leaf extract is used in the treatment of bilharziasis and it is given to pregnant women in Niger as an oxytocic[8]. The root and the stem bark aqueous extracts are used as antidote to snake bite and as arrow poison[9,10]. The root decoction, boiled along with *Hibiscus sabdariffa*, is used for the treatment of syphilis. The root decoction with *Daniellia oliveri* is also used in wound treatment. The gum resin is used along with other medicines as a stomachic and for the treatment of venereal diseases[9]. When burned the stem bark serves as a fumigant and deodorant[10]. The use of the stem bark of *Boswellia dalzielii* to treat fever, rheumatism and gastrointestinal disorders has also been reported[11,12]. The stem bark is boiled in large quantities to make a wash for septic sores. It also serves as part of a multi-component prescription for treating leprosy[10-13]. In Nigeria (Adamawa state), the fresh bark is eaten to induce vomiting and to relieve symptoms of giddiness and palpitations. *Boswellia carteri* Birdw has been shown to have weak antioxidant activity[14] and it is reported that boswellic acids of *Boswellia serrata* Roxb exhibited anticancer activity in different types of cancer like, prostate cancer, skin cancer, brain tumour and blood cancer[15].

WHO recommends the use of plant-based medicines as an alternative medicine, especially in developing countries[14]. Plants also contain a variety of phytochemicals such as phenolics and flavonoids, which provide important health benefits[16]. Antioxidants play a very important role in protecting the body against oxidative stress and free radical damage, which cause various ailments including diabetes,

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heart disease, cancer, brain dysfunction and weakened immune system. In a study done on the antioxidant activity of many plant extracts, it was found that phenolic and flavonoid compounds are mainly responsible for the antioxidant and free radical scavenging effect of the plants[12]. Intake of sufficient amounts of antioxidants is necessary to prevent free radical-induced oxidative stress[16].

Although some biological activities of *Boswellia dalzielii* stem bark extracts have been reported, such as antibacterial[2,11], antifungal[17], hypoglycemic[5], larvicidal[6], with little effect on rat liver function[7] and testes[4]; there is no record in literature of its quantitative antioxidant capacity and antiproliferative activity. Therefore, the present study was conducted to determine total phenolic content, total flavonoid, antioxidant and antiproliferative activities of the stem bark of *Boswellia dalzielii*.

MATERIALS AND METHODS

Collection and identification of plant

The plant material was collected in Jos, Plateau State, Nigeria during the dry season (between December and March 2015) and was authenticated by comparing with voucher specimen (Number: UJ/PCG/HSP/89B13), deposited at the Herbarium of the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Jos, Nigeria.

Preparation of extract

The bark of *Boswellia dalzielii* collected was chopped into small pieces, air-dried to obtain the dried plant material, and powdered in a wooden mortar. The dried, powdered plant material (1 kg) was soaked in about 4 litres of 70% methanol. This was filtered after 24-hour maceration. The filtrate obtained was evaporated to dryness on a vacuum rotary evaporator to yield 236 g (23.6 %) of crude extract.

Solvent partitioning of the methanol extract

The crude methanol extract (100 g) was suspended in water and partitioned exhaustively with hexane (5 x 200 mL) in a separatory funnel. The hexane layer being pooled together and the aqueous layer was also partitioned with ethyl acetate (5 x 200 mL) until clear solvent layer was obtained. The ethyl acetate portions were similarly pooled together. The resultant hexane, ethyl acetate and aqueous fractions were concentrated to dryness on a rotary evaporator and their yields noted as 2.2457 g (0.22%); 17.4318g (1.17%) and 803225 g (8.03%) respectively. The fractions obtained were stored in a desiccator for further work.

Accelerated Gradient Chromatography (AGC) of ethyl acetate fraction

Ethyl acetate fraction (15 g) was subjected to Accelerated Gradient Chromatography using hexane (100%), hexane-ethyl acetate (1:1), ethyl acetate (100%), ethyl acetate-methanol (1:1), and methanol (100%) solvent systems. This gave four (4) sub-fractions noted as A, B, C and D which were concentrated to dryness and their weights noted. Analytical thin-layer chromatographic analyses of the fractions were carried out on commercial pre-coated TLC aluminium plate of Silica gel GF_{254} using ethyl acetate-hexane-chloroform-methanol (1:2:3:4). After development, the plates were sprayed with anisaldehyde and subsequently heated for 5 min. in an oven maintained at 100°C. The coloured spots were noted and their R_f values recorded. Similar spots were bulked based on the R_f values, intensity, colour and size of the spots.

Table 1: Phenolic and flavonoid content of extract, fractions, and sub-fractions of *Boswellia dalzielii* stem bark

Samples	TPC (mg GAE/g extract)	TFC (mg RE/g extract)
Methanolic extract	373.9 ± 27.2	142.2 ± 4.8
Aqueous fraction	318.8 ± 33.4	130.6 ± 9.8
Ethyl acetate fraction	356.4 ± 28.0	112.9 ± 8.2
Hexane fraction	157.6 ± 13.9	123.8 ± 14.4
Sub-Fraction A	125.0 ± 7.7	63.7 ± 1.1
Sub-Fraction B	339.2 ± 13.4	71.7 ± 2.9
Sub-Fraction C	481.2 ± 10.1	129.9 ± 10.7
Sub-Fraction D	331.8 ± 7.8	97.5 ± 5.9

Values are expressed as mean ± SD (n=3). GAE-Gallic acid equivalents; RE-Rutin equivalents, TPC-Total phenolic contents, TFC-Total flavonoid contents.

Table 2: DPPH (IC_{50}) and FRAP (EC_{50}) radical scavenging activity of *Boswellia dalzielii* stem bark

Samples	DPPH scavenging activity (IC_{50})	FRAP scavenging activity (EC_{50})
Vitamin C*	0.39	-
Rutin**	1.99	1.12
Gallic acid***	10.00	-
Methanolic extract	1.99**	4.78**
Hexane fraction	50.11*,**,***	120.22**
Ethyl acetate fraction	3.16**	5.62**
Aqueous fraction	1.58**,***	1.00**
Sub-fraction A	501.10*,**,***	251.18**
Sub-fraction B	6.31**,***	11.22**
Sub-fraction C	5.01**,***	3.98**
Sub-fraction D	7.94**,***	1.25**

Key: asterisks indicate $p < 0.05$ and they are statistically significant.

Total phenolic content determination

Total phenolic content of the extracts were evaluated by a colorimetric method utilizing Folin-Ciocalteu reagent according to the method described by Adedapo[18]. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent thereby producing blue coloured complex. The phenolic concentration of extract, fractions and sub-

Table 3: Anti-proliferative activities of *B. dalzielii* stem bark extract and fractions

Treatment	Conc (µg/ml)	No. germinated (Mean±SEM)	% Seed germination	% Inhibition
Methanolic extract	500	14.66 ± 0.88	70	30
Methanolic extract	250	17.66 ± 0.33	85	15
Methanolic extract	125	15.66 ± 1.45	75	25
Aqueous fraction	500	14.00 ± 2.08	70	30
Aqueous fraction	250	16.33 ± 0.66	80	20
Aqueous fraction	125	16.00 ± 2.00	80	20
Ethyl acetate fraction	500	17.66 ± 0.66	85	15
Ethyl acetate fraction	250	16.33 ± 1.76	80	20
Ethyl acetate fraction	125	17.00 ± 0.57	85	15
Hexane fraction	500	19.00 ± 0.57	95	5
Hexane fraction	250	18.66 ± 0.88	90	10
Hexane fraction	125	19.33 ± 0.66	95	5
Sub-fraction A	500	18.33 ± 0.88	90	10
Sub-fraction A	250	19.66 ± 0.33	95	5
Sub-fraction A	125	20.00 ± 0.00	100	0
Sub-fraction B	500	15.00 ± 2.08	75	25
Sub-fraction B	250	10.66 ± 0.33	50	50
Sub-fraction B	125	11.33 ± 0.33	55	45
Sub-fraction C	500	3.00 ± 1.52	15	85
Sub-fraction C	250	7.00 ± 1.52	35	65
Sub-fraction C	125	2.66 ± 0.33	10	90
Sub-fraction D	500	4.33 ± 0.88	20	80
Sub-fraction D	250	8.66 ± 1.33	40	60
Sub-fraction D	125	2.00 ± 0.57	10	90
Water (control)	-	20.00 ± 0.00	100	0

fractions was evaluated from a gallic acid calibration curve. Aliquots (500 µL each) of 10, 20, 30, 40, 50, and 60 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. The tubes were vortexed for 10 s and allowed to stand for 2 h at 25ºC. After incubation at 25°C for 2 h, absorbance was measured at 765 nm against reagent blank on spectrophotometer (Shimadzu UV-1650, Japan). Total phenolic content was expressed as mg gallic acid equivalent/g. All determinations were performed in triplicate[18].

Total flavonoid content determination

The total flavonoid content of the extract was measured by employing aluminium chloride colorimetric assay reported by Huma[19]. Aluminium chloride colorimetric method was used. The plant extract (1 mL) was diluted with 4 mL of distilled water in a 10 mL volumetric flask. To the mixture, 0.3 ml of 5% $NaNO_2$ was added. At the 5th minute 10% $AlCl_3$ (0.3 ml) and at 6th minute 2 mL of NaOH (1 M) were added. The volumetric flask was made up to the 10 mL mark by addition of distilled water and mixed well. Absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer (Shimadzu UV-1650, Japan). The total flavonoid content in the plant extract was expressed as milligram of rutin equivalents (RE) per g of extract. All the determinations were carried out in triplicate[19].

DPPH radical scavenging assay

The antioxidant activity (free radical scavenging activity) of the extract, fractions and sub-fractions (A, B, C, and D) relative to the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) were determined according to the method reported by Odumosu[20]. The following concentrations of extract were prepared in methanol: 500, 250, 125, 62.50, 31.25, 15.62, 7.81, 3.91, 1.95 and 0.98 µg/mL. Each concentration (2 mL) was mixed with 4 mL of 50 µM DPPH solution in methanol and prepared in triplicate. The mixture was vortexed for 10 s to homogenize the mixture and test tubes were incubated for 30 min at room temperature in the dark. Absorbance was measured at 515 nm on a spectrophotometer (Shimadzu UV-1650, Japan). Lower absorbance readings of the reaction mixture indicate higher free radical scavenging activity. Gallic acid, ascorbic acid and rutin were used as standards at the following concentrations 100, 50, 25, 12.5, 6.2, 3.1, 1.5, 0.7, 0.3, & 0.1 µM. Blank solutions were prepared by mixing 2 mL of methanol with 4 mL of 50 µM DPPH solutions in methanol. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as percentage scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\%\ \text{of antioxidant} = \frac{Ab-As}{Ab} x\ 100$$

where Ab = absorbance of blank; As = absorbance of the sample

Finally, the IC_{50} value, defined as the concentration of the sample leading to 50% reduction of the initial DPPH concentration, was calculated from the separate linear regression plots of the mean percentage of the antioxidant activity against concentration of the test extract (µg/mL)[20].

Ferric Reducing Antioxidant Power (FRAP) assay

The FRAP assay was used to estimate the reducing capacity of the stem bark of *Boswellia dalzielii* extracts, according to the method reported by Kalava & Menon[21]. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3$. $6H_2O$ and 25 ml of 300 mM acetate buffer (pH 3.6). It was freshly prepared and warmed to 37°C. FRAP (900 µL) reagent was mixed with 90 µL water and 30 µL of the extract. The reaction mixture was incubated at 37°C for 30 minutes and the absorbance was measured at 593 nm on a spectrophotometer (Shimadzu UV-1650, Japan). The values are expressed as mmol $FeSO_4$ equivalents per gram of sample. The measurements were done in triplicate[21].

Determination of antiproliferative activity with Sorghum bicolor seeds.

The method of Ikpefan and Ayinde[22] was used for determination of antiproliferative activity. *Sorghum bicolor* seeds obtained from Terminus market, in Jos, Plateau State, Nigeria was cleansed with absolute alcohol for 1 minute and finally with distilled water and air-dried before use. The viability of the seeds was determined by their ability to sink in water. Those that remained submerged in water were removed and dried for use. The respective *B. dalzielii* extracts and fractions were prepared in distilled water at concentrations of 500, 250 and 125 µg/mL were, poured into 9 cm wide Petri dishes laid with cotton wool and filter paper (Whatman No 1). Twenty (20) seeds of sterilized *Sorghum bicolor* were spread in sterile Petri dishes containing cotton wool. The Petri dishes were incubated at room temperature in the dark for 96 hours. Thereafter the number of seeds germinated in each Petri dish was counted and recorded. The control seeds were treated with distilled water only, containing no extracts. The experiments were carried out in triplicate while statistical analyses were carried out using SPSS (version 20). The percentage of seeds germinated at 96 hours was calculated as:

$$\% \text{ seed germination} = \frac{NGS}{TNSU} x\ 100$$

NGS = number of germinated seeds TNSU = total number of seeds used

The percentage of inhibition of seed germination at 4 days was calculated as:

% inhibition of seed germination = 100- % seed germination[22]

Statistical analysis

All data were analysed by standard computer program SPSS (version 20.) and are expressed as mean ± S.E.M. Significant differences were evaluated using Student's t-test. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

The amounts of phenolics (TPC) in the extract, fractions and sub-fractions of *Boswellia dalzielii* bark are reported, as mg of gallic acid equivalent (GAE) per g, while the amounts of flavonoids (TFC) are given as mg of rutin equivalent (RE) per g of extract, fractions and sub-fractions (Table 1).

The methanolic extract had the highest content of phenolics and flavonoids when compared to the major fractions, whereas the hexane and ethyl acetate fractions had the lowest TPC and TFC respectively. It was observed that, of all extracts and sub-fractions, sub-fraction C had the highest TPC (481.20 ± 10.13 mg GAE/g) whereas sub-fraction A has the least (125.04 ± 7.73 mg GAE/g). Methanolic extract of *Boswellia dalzielii* stem bark has the highest TFC (142.17 ± 4.82 mg RTE/g) while the least TFC was recorded for sub-fraction A (63.71 ± 1.06 mg RE/g). Considering the close correlation between TPC/TFC and antioxidant activity, it is expected that the trend observed for TPC/TFC will be reflective of antioxidant activities of the extract and fractions.

Several methods can be used to evaluate antioxidant activity. One that is rapid and widely used is to evaluate the radical scavenging activity of the test substance using DPPH, a stable free radical[23]. Another method, FRAP assay, was employed in this study to complement the DPPH assay. The results are presented in Table 2 as IC_{50} and EC_{50} of extract, fractions and sub-fractions of *Boswellia dalzielii* stem bark.

The methanolic extract, ethyl acetate and aqueous fractions of this plant exhibited significant antioxidant potential when comparing their IC_{50} with the IC_{50} of the standard vitamin C, gallic acid and rutin. Sub-fractions B, C and D showed better antioxidant activities when compare to standard gallic acid in the DPPH assay. The aqueous fraction exhibited highest antioxidant activity (lowest IC_{50}) even higher than gallic acid and rutin, and possessed the highest DPPH radical scavenging activity among all fractions and subs-fractions. Sub-fraction A with an IC_{50} above 500 µg/ml, exhibited the least DPPH radical scavenging activity. The DPPH radical scavenging activity of *Boswellia dalzielii* stem bark may be attributed to the presence of phenolic and flavonoids contents. This can be seen in the good correlation between TPC/TFC and antioxidant activities. The phenolic constituents found in vegetables and spices have received considerable attention due to their antioxidant activity. The antioxidant activity of phenolic constituents has been attributed to their oxido-reduction properties, which play an important role in the adsorption and neutralization of free radicals[24]. The methanolic extract, fractions and sub-fractions showed antioxidant activities because the solvent used has a high affinity for extracting phenolics and flavonoids, while the less polar hexane has a lower affinity for the extraction of these compounds. This quantitative antioxidant activity confirm the results obtained by Alemika et al.[3] who demonstrated the qualitative antioxidant activity of stem bark of *Boswellia dalzielii.* The gum resin of *Boswellia dalzielii* had also been shown to have significant antioxidant activity[25].

In the FRAP assay, ferric ions are reduced to ferrous ions in the presence of an antioxidant (or, a reducing agent) which then forms blue-coloured ferrous tripyridyltriazine

complex (Fe^{2+}-TPTZ) at pH 3.6. The change is monitored spectrophotometrically at 593 nm[26]. The aqueous fraction exhibited the highest FRAP antioxidant capacity with EC_{50} 1.00 µg/ml followed by methanolic 4.78 µg/ml, ethyl acetate 5.62 µg/ml and hexane fraction 120 µg/ml. Of all sub-fractions, the highest activity was observed in sub-fraction D with EC_{50} 1.25 µg/ml, followed by sub-fractions C, B and A (3.98 µg/ml, 11.22 µg/ml and 251.18 µg/ml respectively). Activities observed for aqueous fraction and sub-fraction D were the same with standard antioxidant rutin 1.12 µg/ml. The FRAP assay activity observed for extracts and sub-fractions gave values comparable with that of the standard antioxidant, rutin.

Results of antiproliferative activity are expressed as percentage germination and percentage inhibition of *Sorghum bicolor* seeds in the presence of the extract, fractions and sub-fractions of *Boswellia dalzielii* stem bark (Table 3).

This method is of tremendous value because it is simple, rapid, reproducible, time and material saving. It can be carried out in laboratories where appropriate human cell lines are not readily available. The methods can be used to screen and identify medicinal plants that are claimed to treat tumour related ailments. Research work into natural products with probable antitumor effects can benefit from the use of such simple bench-top bioassay models[22]. From the results of tests for antiproliferative activity it was observed that sub-fractions C and D at a dose of 125 µg/ml has highest activity with 90% inhibition of seed germination at 96 hours, hexane fraction and sub-fraction A at different doses of 250 µg/ml and 500 µg/ml respectively give the same percentage inhibition (10%). This result indicates that the plant *Boswellia dalzielii* has antiproliferative activity, which resides in the sub-fractions C and D. The results are similar to those obtained by Ojerinde[25] on antiproliferative activity of *Boswellia dalzielii* gum resin. The results of antioxidant and antiproliferative evaluation from this study further validate the ethnomedical application of the plant in the treatment of liver disease, brain dysfunction and cancer among others. It also shows the similarity of *B. dalzielii* stem bark in activity with its congeners such as *B. carteri* and *B. serrata*[27].

CONCLUSION

Extracts and sub-fractions of *Boswellia dalzielii* Hutch stem bark showed high TPC and TFC. The phenolic and flavonoid compounds in the extract and fractions are responsible for the antioxidant activity. This is evident from the high antioxidant activity of the stem bark extract and fractions in the DPPH and FRAP assays. The extracts and fractions also showed good antiproliferative activity. Thus, the stem bark of *Boswellia dalzielii* could be useful in disease conditions involving oxidative stress and cancer. Both antioxidant and antiproliferative activities are more pronounced in sub-fractions C and D, showing that they can be further processed to obtain bioactive compounds.

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