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Antifungal evaluation and phytochemical screening of methanolic extract and fractions of *Boswellia dalzielii* stem bark

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Abstract
The objective of the study was to further examine the medicinal value of *Boswellia dalzielii* plant by evaluating the antifungal activity and carrying out phytochemical screening of methanolic extract, hexane, ethyl acetate, aqueous fractions and the sub-fractions of the stem bark of the plant. Standard methods were used for the evaluation of phytochemical screening. Seeded plate method was used for the antifungal test procedures. The phytochemical screening of the stem bark of *B. dalzielii* indicated the presence of tannins, saponins, flavonoids, steroids, carbohydrate. The methanolic extract, fractions and sub-fractions showed activity against *Candida albicans*; ethyl acetate and aqueous fractions showed activity against *Penicillium notatum*, while the methanolic extract, fractions and sub-fractions had no activity against *Aspergillus niger*. These results underscore the importance of *Boswellia dalzielii* as a potential source of antifungal agents.

Keywords: Antifungal; *Boswellia dalzielii*; Phytochemical; Stem bark

INTRODUCTION

Many plants in Africa have been used as sources of remedies for many diseases. Africans are still keeping this old tradition of using plant extracts as remedies for their health needs [1]. WHO recommends the use of plant-based medicines as alternative medicines, especially in developing countries [2]. Medicinal plants produce nutritive and non-nutritive compounds which show antimicrobial activities and protect from pathogens. Thus, it is important to characterize medicinal plants for their antimicrobial activities. Use of plant extracts for their antimicrobial activity has become very important in the light of the increasing threat of global antimicrobial resistance. Fungi are pathogenic microorganisms causing a number of infections of skin, nail or hair, minor infections of mucous membranes or systemic infections causing progressive often fatal disease [3].

Boswellia dalzielii Hutch is a plant from the genus *Boswellia* and the family of Burseraceae. It is a tree plant that is commonly found in North-Western Nigeria

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and often used, among the local population as a source of ethno-medicine [4-7]. In Cameroon, people use the leaves of *B. dalzielii* to protect maize, millet and *Sorghum* against weevil attacks [8]. 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 [9]. The leaf extract is used in the treatment of bilharziasis and it is given to pregnant women in Niger as an oxytocic [10]. The root and the stem bark aqueous extract are used as antidote to snake bite and as arrow poison [11,12]. The root decoction, boiled along with *Hibiscus sabdariffa,* is used for the treatment of syphilis. The root decoction with *Daniella oliveri* is used in wound treatment. The gum resin is used along with other medicines as a stomachic and for the treatment of venereal diseases [11]. When burned the stem bark, serves as a fumigant and deodorant [12]. The use of the stem bark of *Boswellia dalzielii* to treat fever, rheumatism and gastrointestinal disorders has also been reported [13,14]. 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 [12,15]. In Nigeria (Adamawa state), the fresh bark is eaten to induce vomiting and to relieve symptoms of giddiness and palpitations. *Boswellia carteri* Birdw has shown weak antioxidant activity [2] 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 tumor and blood cancer [16].

Among various medicinal uses of the stem bark of *Boswellia dalzielii* reported, there is no record in the literature about antifungal activities. Therefore, the study was conducted to evaluate the phytochemical and antifungal activity of the stem bark of *Boswellia dalzielii*.

EXPERIMENTAL

Plant material. The plant material was collected from 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, Jos Nigeria.

Microorganisms. Three fungi; *Aspergillus niger, Penicillium notatum* and the yeast *Candida albicans* were used in this experiment. These were obtained from stock cultures of the Microbiology unit of the Department of Pharmaceutics and Pharmaceutical Technology, University of Jos, Nigeria.

Plant extraction. The bark of *Boswellia dalzielii* collected was chopped into small pieces and air-dried to obtain the dried plant material. A 1 kg sample of the dried plant material was soaked in 3.7 litters of methanol-70%. This was filtered after 24 h (maceration). The filtrate obtained was evaporated to dryness using Bibby vacuum rotary evaporator (RE 100). The dried extract obtained (236.04g, 23.60 %) was stored in a desiccator.

Organic solvent partitioning. The methanol extract (100g) was suspended in water and partitioned exhaustively with *n*-hexane (5 X 200 mL) in a separating funnel. The hexane layer was allowed to separate, collected and pooled together. The partitioning was continued with ethyl acetate and this was repeated until a clear lower layer was obtained. The hexane, ethyl acetate and aqueous fractions were concentrated to dryness on a rotary evaporator and their respective yields noted. After concentrating to dryness, 2.2457g (0.22%) of hexane, 17.4318g (1.17%) of the ethyl acetate and 803225g (8.03%) of the aqueous fractions

were obtained kept in a desiccator for further use.

Accelerated Gradient Chromatography (AGC) of ethyl acetate fraction. Ethyl acetate fraction (15g) was subjected to accelerated gradient chromatographic procedure using eluents, hexane (100%), hexane-ethyl acetate (1:1), ethyl acetate (100%), ethyl acetate-methanol (1:1), methanol (100%). Chromatographic fractions were collected in test tubes and monitored by silica thin layer chromatography (TLC). Based on the TLC pattern, four sub-fractions coded A, B, C and D which were obtained. These were concentrated to dryness and their weights taken.

Phytochemical screening. Qualitative tests for the presence of plant chemical constituents in were carried out on the methanol extract and fractions using standard procedures [17].

Antifungal screening. The antifungal screening of the plant extract, fractions and sub-fractions were determined by using seeded plates method. Pure cultures of three fungal organisms were used (*Aspergillus niger, Penicillium notatum and Candida albicans*). Antifungal screening of the extract, fractions and sub-fractions were carried out by the Sabouraud Dextrose Agar (SDA) seeded plate method, SDA was prepared and sterilized before being mixed with the organisms, and this mixture was poured into sterile Petri dishes that were previously autoclaved. Using a sterile corn borer (6 mm) three wells were bored at equal distances around each plate and the fourth well was made in the middle. The concentrations of extract, fractions and sub-fractions at 100 mg/mL, 50 mg/mL and 25 mg/mL were then put in the wells using micro pipettes. The control (2 mg/mL fluconazole) was put in the fourth well made in the middle. This was kept on the bench for one hour to allow the extract diffuse. The plates were then incubated for four days at 25^0C. Zone diameters were measured with the aid of a millimetre ruler. The study was carried out in triplicates.

Statistical analysis. All data were analysed by SPSS statistics T-test (version 20) and expressed as Mean ± SD.

RESULTS AND DISCUSSION

The phytochemical screening of the stem bark indicates the presence of carbohydrates, flavonoids, saponins, steroids and tannins in moderate proportion while alkaloid and anthraquinones were absent. This result is in agreement with those obtained by previous workers [7,18-20], on the stem bark of *Boswellia dalzielii*. Some works reported that medicinal plants may contain many species of chemical components and their biological activities are not due to a single moiety. The presence of these constituents gives an indication of the medicinal value of the stem bark. Tannins have the ability to decrease bacteria cell proliferation by blocking key enzymes of microbial metabolism. Flavonoids have also been found to possess antimicrobial properties [20]. The presence of these constituents in stem bark of *Boswellia dalzielii* validates the claims by traditional healers. The results of the antifungal screening of the extract and some fractions are presented in Table 2. On the basis of the zones of inhibition, the aqueous fraction demonstrated higher antifungal activity against *Candida albicans* than other fractions. The ethyl acetate fraction inhibited *Candida albicans*, followed by methanolic extract and hexane fraction. For the sub-fractions, sub-fraction B showed good activity against *Candida albicans*, followed by sub-fractions D and A. All these extract, fractions and sub-fractions were compared with the standard drug, fluconazole.

Table 1. The phytochemical screening of the extract, fractions and sub-fractions of the stem bark of *Boswellia dalzielii*.

Metabolite	Samples							
	Methanol extract	Ethyl acetate fraction	Hexane fraction	Aqueous fraction	Sub-F A	Sub-F B	Sub-F C	Sub-F D
Alkaloids	-	-	-	-	-	-	-	-
Saponins	+	+	-	+	-	-	+	+
Tannins	+	+	-	+	-	+	+	+
Flavonoids	+	+	-	+	-	+	+	+
Steroids	+	+	+	-	+	+	-	-
Anthraquinones	-	-	-	-	-	-	-	+
Carbohydrate	+	+	+	+	+	+	+	+

Key: (+) mean presence of metabolite (-) Means absence of metabolite. Sub-F = Sub-fraction

Table 2: The antifungal activity of the extract, fractions and sub-fractions of the stem bark of *Boswellia dalzielii*.

(a) Methanolic extract

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	32.00 ± 8.00	29.33 ± 3.05	23.33 ± 4.16	44.66 ± 7.57
P. notatum	0.00	0.00	0.00	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(b) Hexane fraction

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	20.66 ± 1.15	24.66 ± 5.03	22.00 ± 3.46	44.66 ± 7.57
P. notatum	0.00	0.00	0.00	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(c) Ethyl acetate fraction

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	33.33 ± 7.02	29.33 ± 6.11	16.00 ± 14.42	44.66 ± 7.57
P. notatum	25.33 ± 5.03	26.33 ± 4.04	21.33 ± 2.31	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(d) Aqueous fraction

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	36.00 ± 5.29	28.00 ± 8.00	24.00 ± 4.00	44.66 ± 7.57
P. notatum	24.33 ± 4.04	22.66 ± 3.05	14.66 ± 12.86	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(e) Sub-fraction A

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	24.00 ± 4.00	13.53 ± 11.94	6.66 ± 5.77	44.66 ± 7.57
P. notatum	0.00	0.00	0.00	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(f) Sub-fraction B

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	38.66 ± 4.16	28.13 ± 7.38	23.53 ± 5.85	44.66 ± 7.57
P. notatum	0.00	0.00	0.00	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(g) Sub-fraction C

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	28.00 ± 6.93	28.00 ± 2.00	24.00 ± 5.29	44.66 ± 7.57
P. notatum	0.00	0.00	0.00	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

(h) Sub-fraction D

Test organism	100 mg/mL	50 mg/mL	25 mg/mL	Positive control
Candida albicans	27.33 ± 4.16	23.33 ± 4.16	14.66 ± 12.86	44.66 ± 7.57
P. notatum	0.00	0.00	0.00	35.33 ± 12.85
Aspergillus niger	0.00	0.00	0.00	38.66 ± 9.01

There was no activity shown by methanol extract and sub-fractions against *Penicillium notatum*; but ethyl acetate and aqueous fractions showed activity against *Penicillium notatum*.

The results of this study showed that the crude extract, fractions and sub-fractions of the stem bark of *Boswellia dalzielii* has activity against *Candida albicans* and only ethyl acetate and aqueous fractions has activity against *Penicillium notatum* fungal. Other results obtained from this plant *Boswellia dalzielii* showed that the plant has been reported to have activity against some Gram-positive and Gram-negative bacteria [4,13,20,21]. The results show that the extract, fractions and sub-fractions had substantial inhibitory effect at higher concentrations.

The antifungal activities of the extract, fractions and sub-fractions of stem bark of *Boswellia dalzielii* could be attributed to flavonoids and tannins, which were detected in the phytochemical screening (Table 1) and also noted in other studies [20,22]. However, no activity was recorded for the methanol extract, fractions and sub-fractions of stem bark of *Boswellia dalzielii* against *Aspergillus niger*.

Conclusion. The results obtained in this study on phytochemical and antifungal screening of the stem bark of *Boswellia dalzielii*, indicate that the stem bark of this plant has antifungal activities. This justifies the use of the stem bark of the plant in traditional medicine for the treatment of various diseases caused by microbes. Further studies are being carried out to characterise and identify the compounds responsible for the observed antifungal activity.

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