IJABR Vol. 2(2):81 - 87 (2010)

Original Article

PHYTOCHEMICAL AND ANTIBACTERIAL PROPERTIES OF METANOLIC AND AQUEOUS EXTRACTS OF *Boswellia dalzielii* STEM BARK

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Submitted: Dec 2, 2010; Accepted: Jan. 03, 2011; Published: Feb. 20, 2011

ABSTRACT

Qualitative screening of *Boswellia dalzielii* stem bark demonstrated the presence of tannins, saponins, alkaloids, flavonoids, phenoids, volatile oils, steroids, reduced compound sugars, carbohydrate and cardiac glycosides. The antibacterial activities of methanolic and aqueous extracts of *Boswellia dalzielii* Hutch, against some human pathogenic bacteria was determined *in vitro* using disc diffusion and minimum inhibitory concentration (MIC) method. The stem bark methanolic extract of 10 µl in 20 mg/ml (200 µg disc-1) displayed a potential antibacterial activity against the tested gram-negative bacteria: *Pseudomonas aeruginosa* and *Salmonnella typhi*, and gram-positive bacteria: *Staphylococcus aureus* and *Bacillus subtilis*. From the results obtained, methanolic extract has greater antibacterial activity than aqueous extract. The zones of inhibition for stem bark aqueous and methanolic extract were 0.66 to 6.66 mm and 1.5 to 11.16 mm respectively. This suggests that *Boswellia dalzielii* stem bark contains biocomponents whose antibacterial activities against the tested gram-negative and gram-positive bacteria can be exploited for pharmaceutical use.

Keywords: *Boswellia dalzielii, In-vitro,* Minimum inhibitory concentration (MIC)

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INTRODUCTION

Plants have been used for the treatment of diseases all over the world before the advent of modern clinical drugs, and are known to contain substances that can be used for therapeutic purposes or as precursors for the synthesis of useful drugs. According to Ayepola and Adeniyi 2008, over 50 % of modern drugs are of natural product origin, and as such these natural products play important roles in drug development in pharmaceutical industries. Pytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. It is known that plants produce these to protect themselves but researches have demonstrated that they can protect humans against diseases (Kumar *et al.*, 2009). *Boswellia dalzielii* (Burseraceae) is a tree plant abundantly found in north-western Nigeria, where the Hausa speaking people refer to it as *Hano* or *Harrabi.* This plant is very popular among the locals as a potent source of ethnomedicine. The extract from its leaves is used for the treatment of diarrhoea in poultry. The root decoction of *B. dalzielii* and *Daniella oliveri* is used for wound healing (Etuk *et al.*, 2006a). The fresh stem bark is eaten to induce vomiting and relieve symptoms of giddiness and palpitations. The root decoction of the plant boiled along with *Hibiscus sabdariffa* is used for the treatment of syphilis. The fragrant gum resin from the plant is

used locally for fumigation of clothes and houses and as a deodorant (Etuk *et al.*, 2006b). Oil from the leaves of *Boswellia dalzielii* was found to exhibit significant activity against *S. aureus*, *B. subtilis* and *C. albicans* (Nwinyi *et al.*, 2004a). The aqueous stem bark extract of *Boswellia dalzielii* was reported to show anti-ulcer activity and reduced gastrointestinal motility (Nwinyi *et al.*, 2004b). It also possesses anti-diarrhoeal effect, which may be related to anticholinergic mechanisms (Etuk *et al.*, 2006c). Crude extracts of the root of this plant have been found to show antibacterial activity against some gram-positive and gram-negative bacteria (Olukemi *et al.*, 2005). Olukemi *et al.*, have however not shown the antimicrobial properties of the stem bark extracts against *Pseudomonas aeruginosa*, *Salmonnella typhi*, and *Bacillus subtilis*. The stem bark aqueous extract has been found to contain phenolic compounds such as protocatechuic acid, gallic acid, and ethylgallate. The frequency of life threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immunocompromised patients in developing countries. There has also been reports that a vast majority of the population, particularly those living in rural areas depend on herbal medicines (Gupta, 1994). These therefore translate to an increased need to authenticate the claim by traditional medical practitioners that the plant-*Boswellia diazielii* has some medicinal properties. Such a medicinal plant if authenticated can be exploited as a source of new chemical substance with potential therapeutic effects.

MATERIALS AND METHODS

Sample Collection

Plant Sample

The stem bark of *Boswellia dalzielii* (Hano) was collected from Sokoto town, north-western Nigeria in the month of May, 2010. The plant was identified at the herbarium of Botany Department of the Faculty of Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

Test Microorganisms

Four bacterial strains were used in this study, among these were two gram-negative namely; *Pseudomonas aeruginosa* and *Salmonella typhi*, and two gram-positive namely; *Bacillus subtilis and Staphylococcus aureus.* The organisms were clinical isolates, obtained, standardized, and stored in the Department of Microbiology, Federal University of Technology, Bosso Campus, Minna, Niger State. The bacteria were grown at 37 °C (in incubator) and maintained on nutrient agar slants at 4 °C.

Sample Extraction

Aqueous Extract of *Boswellia dalzielii* Stem bark

Fifty grams of *Boswellia dalzielii* stem bark powder was placed in 400 ml of distilled water in a round bottom flask and fixed to a reflux extractor via glass adaptor at 100 °C. The refluxing was done for four hours. This was filtered off using muslin cloth into a clean beaker and subjected to steam bath evaporation at 100 °C. The extract obtained was stored in a refrigerator at 4°C (Akueshi *et al.,* 2002a).

Methanolic (100 %) Extract of *Boswellia dalzielii* Stem Bark

Fifty grams of *Boswellia dalzielii* stem bark powder was placed in 200 ml of methanol (100 %) in a round bottom flask and fixed to a reflux extractor via glass adaptor at 65 °C. The refluxing was done for four hours. This was filtered off using muslin cloth into a clean beaker and subjected to steam bath evaporation

at 100 °C. The extract obtained was stored in a refrigerator at 4°C for antibacterial activity test (Akueshi *et al.,* 2002b*).*

Phytochemical Screening
Standard screening tests of the methanolic and aqueous extracts of the stem bark of *B. dalzielii* were carried out for various constituents using the method of Trease and Evans (1983). The extracts were screened for the presence of alkaloids, flavonoids, saponins, tannins, carbohydrates, reduced compound sugars, glycosides, and volatile oils using standard laboratory procedures.

In vitro Antibacterial Test
The *In vitro* antibacterial test was carried out by disc diffusion method (Bauer *et al.,* 1966; Barry, 1980) using 25 µl of standardized suspension of tested bacteria spread on nutrient agar plates. The discs (5 mm in diameter) were impregnated with 10 µl of 20 mg ml^{-1} (200 µg $disc^{-1}$), followed by air-drying and were placed on seeded agar plates. Negative controls were prepared using the same solvents to dissolve the plant extracts. Tetracycline (20 µg disc-1) was used as positive control to determine the sensitivity of bacterial strain. The plates were incubated at 37°C for 24 hrs. Antimicrobial activity was evaluated by measuring the zones of inhibition against the tested bacteria. Each assay was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC)
MIC of four different samples of *B. dalzielii* was determined by two-fold serial dilution method (Chandrasekaran and Venkatesalu, 2004). The dose levels of 20 mg ml^{-1} were serially diluted in a nutrient broth of 5 ml with varying concentrations; 10, 5, 2.5, 1.25 and 0.625 mg ml^{-1}. The test tubes were incubated at 37°C for 24 hrs. Controls were used with the test organisms, using distilled water instead of the plant extract. The least concentration of the samples with no visible growth was taken as the MIC (Adesokan, 2007).

RESULTS

Phytochemical Screening
Qualitative phytochemical screening of both solvents (methanolic and aqueous) of stem bark of *B. dalzielii* showed the presence of tannins, alkaloids, flavonoids, cardiac glycosides, reduced sugar, carbohydrates, phenols, volatile oils and steroids (Table 1).

In vitro Antibacterial Activity
The diameter of zones of inhibition of bacterial growth at varying concentration of stem bark extracts of *Boswellia dalzielii* are shown in Table 2. The methanolic extract of stem bark powder showed stronger antibacterial activity against the studied gram-negative bacteria (*Pseudomonas aeruginosa and Salmonella typhi*) and gram-positive bacteria (*Bacillus subtilis and Staphylococuss aureus*), with the respective diameter zones of inhibition as: 1.50±0.50, 11.16±3.33 and 9.33±3.51, 1.50±1.322 mm.

Aqueous extracts of stem bark powder exhibited a relatively potent inhibitory effect against all the tested gram-negative bacteria (*Pseudomonas aeruginosa and Salmonella typhi*) and gram-positive bacteria (*Bacillus subtilis and Staphylococuss aureus*) and their respective diameter of zones of inhibition were 3.00±2.00, 7.00±2.00, and 6.66±2.08, 0.66±0.288 mm, respectively.

Table 1: Phytochemical Analysis of Aqueous and Methanolic Extracts of *Boswellia dalzeilii* Stem Bark

Phytochemical Components	Aqueous Extract	Methanolic Extract
Saponins	+++	+++
Tannins	+++	+++
Flavonoids	++	+++
Alkaloids	+++	+++
Cardiac glycosides	+++	++
Reduced Sugar	+++	+++
Carbohydrates	+++	+++
Protein	–	–
Amino acid	–	–
Steroids	+++	+++
Phenols	+++	+++
Volatile oil	+++	+++

+++ - Highly present
++ - Moderately present
+ - Trace
– - Absent

Table 2: Antibacterial Activity of *Boswellia dalzielii* Stem bark Extracts against Some Human Pathogenic Bacteria

		Tests organisms			
		Diameter zone of inhibition			
Extracts	Concentration mg/ml	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Salmonella typhi*	*Staphylococcus aureus*
Methanolic extract	20	1.50±0.50	9.33±3.51	11.16±3.33	1.50±1.32
Aqueous extract	20	3.00±2.00	6.66±2.08	7.00±2.00	0.66±0.29
Tetracycline	20	18.30±0.12	7.25±0.08	14.16±0.23	12.76±0.02

* Tetracycline concentration is in µg/disc, Diameter of cork borer = 5 mm
*Values are presented as mean ± Standard Error of triplicate experiments.

Table 3: Minimum Inhibitory Concentration of *Boswellia dalzielii* Extracts on Isolates.

	Concentration (mg/ml)			
Sample	*S. aureus*	*P. aeruginosa*	*S. typhi*	*B. subtilis*
Methanol	10	2.5	2.5	2.5
Aqueous	ND	ND	ND	ND

ND =not detected.

DISCUSSION

The presence of tannins in the methanolic and aqueous stem bark extracts of *B. dalzielii* supports the traditional medical use of this plant in the treatment of many ailments. This is supported by Dharmananda 2003. According to him, herbs that have tannins as their component are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery. In addition to its antimicrobial properties, tannins also have antioxidant properties. Alkaloid showed a marked presence in both extracts of *B. dalzielii* stem bark, and it possesses anti-inflammatory, and anti-anaphylactic properties with consequences of altered immunological status *in vivo* (Ganguly and Sainins, 2001). Furthermore, alkaloids have amazing effect on humans and this has led to the development of the powerful pain killer medications. The flavonoid's ability to scavenge hydroxyl radicals, superoxide anion radicals and lipid peroxy radicals have highlighted many of the flavonoid health-promoting functions in organisms which are important for prevention of diseases associated with oxidative damage of membrane, proteins and DNA(Ferguson, 2001). Saponins are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). All the findings above support the usefulness of *B. dalzielii* in traditional medicaments. The results obtained showed that the methanolic extract of stem bark of *B.dalzielii* had greater antibacterial activity than aqueous extract. This was reflected in the varying zones of inhibition of individual extracts on the pathogens *in vitro*. This is in agreement with previous studies reporting the antimicrobial and inhibitory effects of *Boswellia dalzielii* leaves (Gupta, 2005). *Boswellia dalzelii* extracts has therefore displayed a good inhibitory effect on the investigated bacteria (*B. Subtilis and Salmonella typhi*) and showed a great activity by inhibiting *P. aeruginosa and S. aureus*. It however showed lower zones of inhibition compared to that obtained for *B. subtilis and S. typhi. B. dalzielii* leaf extract has been reported to be effective against some gram-positive bacteria, although gram-negative bacteria have been found to be less susceptible to plant extracts in earlier studies carried out by other researchers (Kuhnt *et al.*, 1994; Afolayan and Meyer, 1995). The lower zones of inhibition obtained in this study means that higher doses of the plant extracts would be required in the treatment of infections or could be used in synergy with any other active plants with known potency against microorganism. This suggests that *Boswellia dalzielii* stem bark extracts contain bio-components capable of mimicking the activities of the control drug-tetracycline. Tetracycline exhibited better inhibitory effect against all the tested gram-negative and gram-positive bacteria, except in *B. subtilis,* where the methanolic extract displayed a better inhibitory effect. The inhibitory activities observed on the investigated bacteria (*P. aeruginosa and S. Aureus)* was also confirmed by Siddhuraju and Becker (2003); Vaghasiya and Chanda

(2007), although with lower zones of inhibition compared to that obtained for *S. typhi.* The lower zones of inhibition obtained for *P. aeruginosa and S. aureus* means that higher doses of the plant extracts would be needed in the treatment of infections caused by these bacteria.

CONCLUSION

The activity of the stem bark extracts against both gram-positive and gram-negative bacteria is the confirmation of the presence of pharmacological active compounds. Thus, *B. dalzielii* Hutch could become a promising natural antimicrobial agent with potential applications in pharmaceutical industry for controlling the pathogenic bacteria. However, if plant extracts are to be used for medicinal purpose, issues of safety and toxicity should be considered.

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