REVISED Original research Articles

IN VITRO ANTIMICROBIAL AND ANTIFUNGAL EFFICACY OF ETHANOL CRUDE STEM BARK EXTRACT OF *BOSWELLA DALZIELLE*

ABSTRACT

The efficacy of *Boswellia dalzielii* (Frankincense) stem bark extract on some bacterial and fungal organisms was evaluated for its in-vitro antimicrobial activities against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Salmonella gallinarium, Aspergillus fumigatus* and *Candida albicans*. The research work was carried out in Biochemistry and Microbiology Laboratories of Federal College of Animal Health and Production Technology, National Veterinary Research Institute, Vom. Well diffusion method was carried out on nutrient agar. MIC, MBC and MFC of the test organisms were carried out on nutrient broth. The phytochemistry revealed the presence of saponin, tannin, flavonoids, cardiac glycosides, steroids, terpens and phenol in ethanol extracts while resin, alkaloid and glycosides were absent in hot water extracts. Alkaloid was also absent in ethanolic extract. The aqueous extract of the plant exhibited neither antibacterial nor antifungal effects against all test organisms used in the study while the ethanolic extract of the plant showed both antibacterial and antifungal effects on the study organisms. The results of this study also showed that the ethanolic extract of *Boswellia dalzielii* stem bark has activity against all bacteria species used in the study (broad spectrum activity). For gram-negative and positive bacteria, *Salmonella gallinarium* and *Staphylococcus aureus* were the most sensitive while *Escherichia coli* and *Streptococcus pyogenes* were the least respectively. *Candida albicans* was more sensitive than *Aspergillus fumigatus*. It was concluded that the test organisms were susceptible to ethanol extracts of the plant and may be good source of antibiotics.

KEY WORDS: *Boswellia dalzielii*, Antibacterial activity, Antifungal activity, Plant extract.

INTRODUCTION

Herbal medicine is the oldest form of medicine known to mankind [1, 2]. It was the mainstay of many early civilization and still the most widely practiced form of medicine in the world today [3]. Many people in developing countries still rely on traditional healing practices and medicinal plants for their daily healthcare needs, in spite of the advancement in modern medicine [4]. Traditional medicine which is widespread throughout the world has been recognized by World Health Organization (WHO) as an essential building block of primary health care. According to reports of World Health Organization, 80% of the world's population relies mainly on traditional therapies which involve the use of plants extracts or their active substance [5]. There is abundant undocumented traditional knowledge of herbal remedies used to treat diseases in most cultures [6]. Different traditional healing practices worldwide are designed for either therapeutic or prophylactic use in human or animal diseases [7, 8]. Several studies carried out in Africa, Asia, Europe, Latin America and North America show that plants are routinely used as remedy for animal diseases [9-14]. Historically, it is documented that humans utilize the same herbal preparations that they use to treat their sick animals [15]. In Nigeria, farmers are known to treat animal diseases with herbs and other

traditional medical practices before the advent of orthodox medicine [16]. Traditional medical and veterinary practices remain relevant and vital in many areas in Nigeria due to absence or inadequate provision of modern medical services particularly in rural areas [17]. Ethno-veterinary medical practice is widespread among herdsmen and native livestock producers in northern Nigeria. Traditional remedies in this area include plant extracts from different plant parts [18]. Herdsmen in non-industrialized nations of the world still use medicinal plants for the treatment of livestock diseases, either due to lack of access to trained veterinarians and high cost of orthodox medicines, or the held belief that herbal remedies are more efficacious [19].

Plants are also potential sources of modern drugs. A recent survey of United Nations Commission for trade and development (UNCTAD) indicated that about 13% of drugs produced within developed countries are derived from plants [20]. Surprisingly, this large quantity of modern drugs comes from less than 15% of the plants, which have been known to have been investigated pharmacologically [21]. Therefore, since there are so many of these naturally occurring substances in plants, it is obvious that the plant kingdom offers better opportunity of providing useful medicinal compounds.

Boswellia dalzielii (family Burseraceae) commonly known as frankincense tree abounds in the Savannah regions of West Africa. Boswellia dalzielii is a tree that belongs to the family of *Burseraceae,* from the genus of Boswellia and species of B. dalzielii. It is about 13m high of the wooden savanna with a pale papery bark peeling and ragged characteristic. It is abundantly found in West Africa in countries such as Ghana, Niger, Ivory Coast, Upper Volta and Northern part of Nigeria, where the Hausa speaking people of Nigeria call it “Hano” or “Ararrabi”. The plant is popular in the Northern part of Nigeria due to its ethno medicinal importance [22]. The plant has several medicinal uses which include the decoction of the stem bark use to treat rheumatism, septic sores, venereal diseases and gastrointestinal ailments [23, 24]. Phytochemical studies of the plant revealed the absence of alkaloids [25], while saponins, tannins, flavonoids, cardiac glycosides, steroids, and terpenes were present [26, 27]. Oil from the leaves of *Boswellia dalzielii* was found to exhibit significant activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Candida albicans* [28]. The methanolic and aqueous extracts showed both antibacterial and antifungal activities [29, 27]. Recent studies of the aqueous extract of the stem bark of *Boswellia dalzielii* showed no antimicrobial activity against all the microbes used however the extract produced some anti-ulcer activity [26]. Furthermore, recent study also revealed incensole to be part of the chemical composition of the stem-bark of *Boswellia dalzielii* [30].

Nowadays, the problem of antimicrobial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [31]. Laboratories of the world have found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms *in vitro* [32]. Unfortunately, development of effective antimicrobial agents has been accompanied by the emergence of drug-resistant organisms due to the irrational and over-use of antibiotics, failure to complete a course of treatment, genetic versatility of microbes and horizontal transfer of resistant genes among bacterial species. All the mentioned factors diminish the clinical effectiveness of antibiotics [33, 34].

In recent time, there has been renewed interest on plants as sources of antimicrobial agents due to their use historically and the fact that a good portion of the world’s population,

particularly in developing countries rely on plants for the treatment of infectious and non-infectious diseases [35]. There is paucity of informations on the antifungal activity of the plant extract and also comparative study on the antifungal and antibacterial activities of the plant extracts. Therefore, the aim of this research is to determine the susceptibility of some bacterial and fungal organisms to the ethanolic and aqueous plant extracts and also to determine the minimum antibacterial and antifungal concentrations of the plant extracts.

PLATE 5: *B. dalzielii* plant

PLATE 5: *BOSWELLIA DALZIELII*(FRANKINCENSE) PLANT

MATERIALS AND METHODS

METHODOLOGY

The Standard qualitative method as described by Sofowora, [36] was used for phytochemical screening of the plant using ethanol and hot water as the solvents in the biochemistry laboratory. Well diffusion and tube dilution methods were used to determine the antimicrobial properties, minimum inhibitory concentration and minimum bactericidal concentrations of the plant extract as described by Cheesbrough, [37] while minimum fungicidal concentrations of the plant extract was determined as described by Picman *et al.* [38].

Molten nutrient agar was prepared and 0.2ml of the organism from the broth culture was inoculated into molten nutrient agar and was poured into plates and was left on the bench to solidify [39]

Six wells were bored using sterile borer. The extract were dispensed into each well using a sterile micropipette at different concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml. Gentamycin and amphoteracin B injections were used as positive

controls for both bacterial and fungal organisms respectively. The plates were incubated at 37°c for 24 hours.

PHYTOCHEMICAL SCREENING

The plant *Boswellia dalzielii* was obtained from National Veterinary Research Institute, Vom, Plateau State, Nigeria. It was identified as *Boswellia dalzielii* by Mr. Okonkwo, a plant taxonomist attached to the Federal College of Forestry, Jos. The powdered stem bark (100g) was filtered and extracted exhaustively with petroleum ether 60-80°C in a Soxhlet apparatus for 24hrs. The marsh was air dried and re-extracted with ethanol. The aqueous and ethanolic extracts were separately evaporated under reduced pressure to give solid residues weighing 10.76g and 21.82g, respectively. The residues were then subjected to phytochemical screening using standard tests to show the different types of phytochemical constituents present in the stem [36, 40-42].

Test for Tannins: 10mls of distilled water was added to 0.5 g of the plant extract and was stirred and filtered. To the filtrate, a few drop of ferric chloride solution was added. Deep green coloration was seen which indicates the presence of tannin.

Test for Alkaloids: 3ml of 1 % aqueous solution of HCL was added to 0.5g of the plant extract on steam bath. It was filtered and divided into 2 test tubes. Few drops of Meyers reagent was added to one of the test tubes and picric solution to the other. Formation of precipitate indicates the presence of alkaloids.

Test for Flavonoids: 0.5g of the extract was dissolved in 2mls of dilute sodium hydroxide.

A few drops of concentrated sulphuric acid were then added. A yellow solution indicates the presence of flavonoids.

Test for Glycoside: 10mls of boiling distilled water was added to 0.5g of the plant extract, stirred thoroughly and filtered. 2ml of the filtrate was dispensed with a few drops of concentrated HCL. Few drops of ammonia solution were added to render it alkaline. 2ml of Benedict's reagent was added to 5 drops of filtrate solution and was boiled. A reddish brown precipitate shows the presence of glycoside.

Test for Saponins: Distilled water was added to 0.5g of the extract inside test tube. Persistent frothing which warmed the tube was an evident for the presence of saponin.

Test for steroids and terpens: 0.1g of the extract was dissolved in 1ml of the chloroform. 1ml of acetic anhydride and 2 drops of concentrated H_2S0_4 were added. A pink colour was noticed which changes to bluish green on standing, indicates the presence of steroids and terpens.

Test for cardiac glycoside: 0.1g of the extract was dissolved in 1ml of glacial acetic acid containing 1 drop of ferric chloride solution. 1ml of concentrated sulphuric acid was also

added. A pink colour which changes to bluish green on standing was an indication for the presence of cardiac glycoside.

SOURCE OF THE ORGANISMS

The organisms were collected from the Central Diagnostic Laboratory of the National Veterinary Research Institute Vom and the work was carried out in college Microbiology Laboratory, Federal College Animal Health and Production Technology Vom, Plateau State. These organisms were *Escherichia coli, Salmonella gallinarum, Staphylococcus aureus, Aspergillus fumigatus, Candida albicans* and *Streptococcus pyogenes.* These are the most common pathogenic organisms in the area of study.

SENSITIVITY TEST USING WELL DIFUSSION METHOD

Molten nutrient agar was prepared and 0.2ml of the organism from the broth culture was inoculated into molten nutrient agar and was pour plated and was left on the bench to solidify [43]. Six wells were created in the agar. The extract was dispensed into each well using a sterile micropipette at different concentrations of 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL. Gentamycin and Miconazole were used as positive controls for both bacterial and fungal organisms respectively. The plates were incubated at 37°C for 24 hours.

DETERMINATION OF MINIMUM INHIBITORY AND MINIMUM BACTERIOCIDAL CONCENTRATIONS

Tube dilution method was used in varying concentration of the liquid media and the extracts in test tubes at 500 mg/mL, 250 mg/mL, 125 mg/mL, 62.5 mg/mL and 31.25 mg/mL were dispensed in tubes, and 0.2ml of the standardized organism was also dispensed in the same tubes. The tubes were incubated at 37°C for 24 hours, positive control were also setup. The least concentration without growth gives the MIC. The MIC is then subcultured into a broth culture tubes that contain no extracts, the lowest concentration that result in no growth of the subcultured is noted which indicated MBC, [37].

DETERMINATION OF MINIMUM FUNGICIDAL CONCENTRATION

The hyphal growth inhibition test was used to determine the antifungal activity of the plant extract against fungal strains as previously described Picman *et al.* [38]. Briefly, dilutions of the test solutions dissolved in vehicle were added to sterile melted PDA at 45^{o}C to give final concentrations of 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml and 31.25mg/ml of plants extracts. The resultant solution was thoroughly mixed and approximately 15 mL was poured onto the petri plate. Plugs of 1 mm of fungal mycelium cut from the edge of actively growing colonies were inoculated in the center of the agar plate and then incubated in a humid chamber at 25^{o}C. Control cultures also received an equivalent amount of vehicle. Three replicates were used for each concentration. Radial growth was measured when the control colonies almost reached 1.5 cm.

RESULTS

Table 1: The phytochemical components of *B. dalzielii* stem bark extracts.

s/n	Phytochemicals	Observations	
		Ethanol	Hot water
1	Saponin	++	+
2	Tannins	++	+
3	Resins	+	_
4	Alkaloids	_	_
5	Flavonoids	++	+
6	Glycosides	+	_
7	Cardiac glycosides	++	+
8	Steroids	++	+
9	Terpens	++	+
10	Phenol	+++	+

Key

_ = absent

\+ = slightly present

++ = moderately present

+++= heavily present

Table 2: Antimicrobial activity of the Hot water extracts

ISOLATES	Concentration in mg/ml						
	500	250	125	62.5	31.25	-ve	+ve
Aspergillus fumigatus	_	_	_	_	_	_	17
Candida albicans	_	_	_	_	_	_	18
Staphylococcus aureus	_	_	_	_	_	_	10
Streptococcus pyogenes	_	_	_	_	_	_	15
Escherichia coli	_	_	_	_	_	_	9
Salmonella gallinarum	_	_	_	_	_	_	12

Key:

-ve = control negative (sterile water)

+ve = control positive (Gentamycin and Miconazole for antibacterial and antifungal respectively)

Table 3: Antimicrobial activity of the ethanol extracts

ISOLATES	Concentration in mg/ml						
	500	250	125	62.5	31.25	-ve	+ve

Aspergillus fumigatus	4	4	3	3	2	_	17
Candida albicans	5	4	3	3	2	_	18
Staphylococcus aureus	10	6	4	4	3	_	10
Streptococcus pyogenes	4	3	2	2	1	_	15
Escherichia coli	9	7	5	2	2	_	9
Salmonella gallinarum	7	6	5	3	3	_	12

Table 4: MIC, MBC AND MFC OF THE EXTRACTS

ISOLATES	MIC (mg/ml)	MBC (mg/ml)	MFC (mg/ml)
Aspergillus fumigatus	125	NA	125
Candida albicans	125	NA	_
S. gallinarum	62.5	62.5	
S. aureus	62.5	62.5	
E. coli	125	125	

S. pyogenes	250	250

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

The phytochemical screening of *Boswellia dalzielii* plants showed that it contains saponnin, tannins, flavonoids, cardiac glycosides, steroids, terpens and phenol in both ethanol and hot water extraction while resins and glycosides were present only in ethanolic extract but absent in aqueous extract. Alkaloids was found to be absent in both hot water and ethanol extraction (Table 1). This is in accordance with Nwinyi *et al.* [28] and Anago *et al.* [44] who reported the presence of tannin among the phytochemical properties of the plant and absence of alkaloid in their aqueous and ethanolic extracts respectively.

Hassan *et al*. [42] also reported the presence of tannins, saponins, flavonoids, cardiac glycosides, steroids and terpenes in methanolic etract of the plant.

The aqueous extract of the plant exhibited neither antibacterial nor antifungal effects against all test organisms used in the study (Table 2). This agreed with the report of Nwinyi *et al.* [28] and Taiwo *et al.* [43] who stated that aqueous extract of the plant has no antibacterial effect.

The ethanolic extract of the plant showed both antibacterial and antifungal effects on the study organisms (Table 3). This also agreed with Olukemi *et al*. [45], Nwinyi *et al*. [28], Noumbo *et al.* [46] who reported that ethanolic extract from *Boswellia dalzielii* have antimicrobial property. According to Campbell [47], the presence of substantial level saponnin, phenols and tannins in an extract encourage antimicrobial properties.

The results of this study showed that the ethanolic extract of the stem bark of *Boswellia dalzielii* has activity against some gram-positive and gram-negative bacteria (broad spectrum of activity) [29]. For gram-negative bacteria (Table 4), *Salmonella gallinarium* was the most sensitive while *Escherichia coli* was the least. For gram-positive (Table 4), *Staphylococcus aureus* was the most sensitive while *Streptococcus pyogenes* was the least. *Candida albicans* was more sensitive than *Aspergillus fumigatus* (Table 4). In general, this herb was more active with bacteria than fungi (Tables 4). This is due to the complex nature of fungal cell wall which makes entry of drugs and other chemotherapeutic agents extremely [48]. Nwinyi *et al.* [28] stated that presence tannin is responsible the antibacterial activity of *Boswellia dalzielii* ethanolic extract. According to Olukemi *et al.* [45], *Staphylococcus aureus* is very sensitive to *Boswellia dalzielii* ethanolic extract and also reported that gram-negative bacteria are less susceptible to the extract than gram-positive. The result of the study also correlated with the

use of the stem bark of *Boswellia dalzielii* by herbal practitioners in Jos to treat gastroenteritis [28].

CONCLUSION AND RECOMMENDATIONS

From the results of the of phytochemical, and antibacterial and antifungal screening of the bark of *Boswellia dalzielii*, the study justifies the use of the bark of the plant in traditional medicine for the treatment of various diseases caused by microbes.

Root, stem and leaves extracts of *Boswellia dalzielii* were recommended to be tried on other microorganisms to ascertain its efficacy. More so, phytotoxicity of *Boswellia dalzielii* should be carried out to determine the possible toxicity of the pharmaco-active ingredients of the plant.

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