ChemSearch Journal 13(1): 76 – 80, June, 2022
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 31/03/2022 Accepted: 12/05/2022
http://www.ajol.info/index.php/csj

Phytochemical and Antimicrobial Potential of Methanol Root Bark Extract of *Boswellia Dalzielii*

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ABSTRACT

Medicinal plants have been playing an essential role in the development of human culture as it gives man food, shelter and medicine since antiquity. This research work aimed at screening for the presence of phytochemicals as well as evaluating the antimicrobial activity of the root bark of *Boswellia dalzielii*. Fresh root bark of the plant was dried, pulverized, extracted using methanol by maceration method, screened for the phytochemicals and evaluated the effect of the extract on some resistant pathogenic microbes using disc diffusion method. The result of the phytochemical study revealed the presence of flavonoids, tannins, saponins, terpenoids, cardiac glycosides and Alkaloids. The susceptibility test of root extract of *B. dalzielii* showed that the extract had antimicrobial effect on *Klebsiella spp., Salmonella typhi, Pseudomonas aeruginosa, Streptococcus pyogenes, Corynebacterium specie* and *Shigella dysenteriae.* The antimicrobial activities presented as diameter of inhibition zones showed high activity value in *Salmonella typhi* (19.67±0.00 mm) and *Streptococcus pyogenes* (18.00±0.00 mm) at extract concentration of 500 mg/ml, while *Klebsiella spp*., and *Shigella dysenteriae* were inhibited least at extract concentrations of 62.5 mg/ml. of *Escherichia coli, Staphylococcus aureus, Basillus subtilis, Candida albicans and Aspergillus niger* were all resistant to the extract. Thus, these findings have scientifically justified the use this plant locally for the treatment of some pathogenic related ailments and has provided a clue for the need to isolate the active ingredient(s) responsible for the biological activity from the root of *B. dalzielii*.

Keywords: Antimicrobial, *Boswellia dalzielii*; Phytochemicals, Root Bark, Screening

INTRODUCTION

The present occurrence of antimicrobial drug resistance by most bacteria has posed an enormous problem and triggered the need for continuous research for better and safe therapeutic agents. The rate of drug resistance by microorganism especially bacteria and fungi is growing out of hand, thereby leading to a global health challenge (Sofowora, 2008).

The global campaigns for the search of new bioactive agents with fewer side effects and greater activities have led to the screening for bioactive compounds in many medicinal plants of the Tropics (Masoko *et al*., 2005) especially *Boswellia dalzielii*. The selection of this specie was based on its application in traditional medicine in Africa and other parts of the world for the treatment of microbial infections.

Boswellia dalzielii commonly called "Frankincense tree." is a very tall tree (more than 13meters high), with a smooth pale brown bark that is particularly separated by papery plates ragged (Ethuk *et al*., 2006). It produces small fragrant and aromatic white flowers. It is popular in the Northern part of Nigeria due to its ethnomedicinal importance. The decocted root bark is used traditionally by the Hausa-Fulanis in Sokoto, Nigeria to treat diabetes (Shinkafi, 2015), the bark is boiled up in large quantity to make a wash for fever, rheumatism etc., and the liquid extract is taken internally for gastrointestinal troubles (Burkill, 1985; Danlami *et al.*, 2015), a cold infusion from the plant is used for snake bite (Burkill, 1985). The fresh bark of the root is eaten in Adamawa State, Nigeria, to cause vomiting after a few hours and thus relieves symptoms of giddiness and palpitations as well as an antidote to arrow-poison (Burkill, 1985; Danlami *et al.*, 2015).

To the best of our knowledge, there is no reported work on the antimicrobial activity of the root bark of *Boswellia dalzielii*. Hence this work was aimed at screening the methanol extract of the root bark of *B. dalzielii* for its phytochemicals and antimicrobial activity against eleven (11) pathogenic microbes that are associated with common infections.

MATERIALS AND METHODS

Sample Collection, Identification and Preparation

Fresh root of *Boswellia dalzielii* were collected from Gulantabar, Song Local Government Area, Adamawa State, Nigeria. The identification of the plant was carried out at the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. Consequently, the sample was cleaned, shade dried, pulverized into powder and packed in a container until further use.

Sample Extraction

The powdered material (400g) was soaked with 85 % methanol (1.5 L) for 72 hrs with occasionally shaking 72 h. Subsequently, another 85 % (500 mL) was added to the mixture and filtered using a muslin cloth as well as with Whatmann filter paper No. 1. The filtrate was concentrated to dryness to obtain the extract which was stored in a desiccator until required for use.

Phytochemical Screening

The methanol extract of the root bark of *Boswellia dalzielii* was screened qualitatively for phytochemical constituents using standard procedures (Evans, 2009).

ANTIMICROBIAL ASSAY

Test Microorganisms

All the organisms used in this study were clinical isolates obtained from Department of Medical Microbiology, College of Medical Sciences, University of Maiduguri, Nigeria. Standard antibiotic (Ciprofloxacin 5 µg/disc,) was used for this study as the positive control.

Preparation of Sample Extract

One gram (1g) of the extract was dissolved in 10 ml (100 mg/ ml) of peptone water to obtain a stock solution. The extract was diluted as 1:10 equivalent to 100 mg /ml and 1:5 dilution equivalent to 50 mg /ml from which 0.2 ml of the 1:10 dilution was dispensed into a bored-agar hole (6 mm diameter with a depth of 5 mm) making a concentration of 20 mg / hole and also 0.2 ml of the 1:5 dilution was poured into the ditch hole bored onto the agar equivalent to 10 mg /hole. (Usman *et al.*, 2007).

Determination of Zone of Inhibition

The method of Vollekovà *et al*. (2001) as modified by Usman *et al*. (2007) was adopted for this study. Thus, four holes were bored on the plates (6 mm diameter) using sterile cork-borer. Then about 0.2 ml of the sample extract was inoculated across the wells and incubated at 37 ^{o}C for 18-24 hrs. After incubation, the average diameter of four readings of the clear zone surrounding the hole was taken as the measure of the inhibitory level of the plant extract against the bacteria/fungi which was recorded as mean ± SEM.

Minimum Inhibitory Concentration (MIC*)*

MIC was determined using the broth dilution technique (Vollekova *et al.*, 2001). The minimum inhibitory concentration was determined from micro-organisms that were sensitive to the extract under study (leaf extract). Equal volume of nutrient broth was dispensed into tubes (bijou bottles) where known concentrations of the methanol extract which was diluted at concentrations ranging from 7.5 mg/ml to 60 mg/ml were prepared.

Minimum Bactericidal Concentration (MBC)

MBC was determined using the broth dilution technique as modified by Usman *et al*. (2007) by assaying the test tubes resulting from MIC determinations. Thus, loop of the content of each test tube was inoculated by streaking on a solidified nutrients agar plate incubating at 37 °C for 18 hours and observed for bacterial growth. The lowest concentration of the subculture with no growth was considered as the MBC.

Statistical Analysis

The data obtained from this study was analysed using Graph pad prism version 8.4.3. The results with p value$\leq$0.05 were considered significant and are presented as mean ± SEM.

RESULTS AND DISCUSSION

Phytochemical Screening of Root Bark Extract of *B. dalzielii*

The phytochemical constituents of the methanol extract of *B. dalzielii* root bark showed the presence of flavonoids, tannins, saponins, terpenoids, cardiac glycosides and alkaloids as contained in Table 1. The result of the phytochemical constituents is shown in Table 2.

Antimicrobial Activity of Root Bark Extract of *B. dalzielii*

The susceptibility test of root extract of *B. dalzielii* showed that the extract had antimicrobial effect on *Klebsiella spp, Salmonella typhi, Pseudomonas aeruginosa, Streptococcus pyogenes, Corynebacterium specie* and *Shigella dysenteriae* (Table 2).

Minimum Inhibitory and Minimum Bactericidal Concentration of the Susceptible Microorganisms

The MIC and MBC values against tested pathogenic microbes is shown in Table 3 and Table 4 respectively.

Table 1: Phytochemical constituent of methanol root extract of *Boswellia dalzielii.*

S/No	Constituent	Test	Inference
1.	Flavonoids	Shinoda	+
		Ferric chloride	+
		Lead acetate	+
		Sodium hydroxide	-
2.	Carbohydrates	Molisch	+
		Monosaccharides	-
		Free reducing sugar	+
		Combined reducing sugar	+
		Ketones	+
3.	Tannins	Ferric chloride	+
		Lead acetate	+
4.	Saponins	Frothing	+
5.	Terpenoids	Terpenoids	+
6.	Cardenolides	Keller-Killiani`s	+
7.	Glycosides	Free anthraquinone	-
		Combined anthraquinone	-
8.	Cardiac glycosides	Salkowski	+
		Liebermann-Burchard	+
9.	Alkaloids	Dragendroffs reagent	-
		Meyer's reagent	+

Key: (+) presence and (-) absence of some phytochemicals

Table 2: Antimicrobial activity of the methanol root bark extract of *Boswellia dalzielii* using Disc diffusion method

	Concentration (mg/ml)/ Zone of Inhibition (mm) in Mean±SEM				
	500	**250**	**125**	**62.5**	**5 (Cip)**
Escherichia coli	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	30.33±0.33
Salmonella typhi	19.67 ± 0.33^{a}	15.67 ± 0.33^{b}	11.33 ± 0.33^{c}	8.33 ± 0.33^{d}	28.33 ± 0.33^{e}
Klebsiella spp	14.67 ± 0.33^{a}	12.00 ± 0.00^{b}	9.00 ± 0.00^{c}	7.00 ± 0.00^{d}	22.00 ± 0.00^{e}
Pseudomonas aeruginosa	14.67 ± 0.33^{a}	11.33 ± 0.33^{b}	8.33 ± 0.33^{c}	0.00 ± 0.00^{d}	34.00 ± 0.00^{e}
Staphylococcus aureus	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	20.33±0.33
Streptococcus pyogenes	18.00 ± 0.00^{a}	14.33 ± 0.33^{b}	11.00 ± 0.00^{c}	8.00 ± 0.00^{d}	25.33 ± 0.33^{e}
Bacillus subtilis	0.00 ± 0.00^{a}	0.00 ± 0.00^{ba}	0.00 ± 0.00^{ca}	0.00 ± 0.00^{da}	18.33 ± 0.33^{e}
Corynebacterium specie	15.00 ± 0.00^{a}	11.00 ± 0.00^{b}	$8.33.\pm0.33^{c}$	0.00 ± 0.00^{d}	19.50 ± 0.00^{e}
Shigella dysenteriae	17.67 ± 0.33^{a}	14.00 ± 0.00^{b}	10.00 ± 0.00^{c}	7.00 ± 0.00^{d}	18.00 ± 0.00^{ea}
Candida albicans	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	11.00±0.00
Aspergillus niger	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NT

Key: Value on the same row with different alphabetical superscript are statistical significant $p<0.05$; n=3; Cip= Ciprofloxacin (Control drug); NT: No treatment; Mean ± SEM.

Table 3: Minimum Inhibitory Concentration (MIC) of methanol root bark extract of *Boswellia dalzielii*

Microorganism	Concentration of Extract (mg/ml)				
	60	**30**	**15**	**7.5**	**3.25**
Salmonella typhi	-	-	-	α	+
Klebsiella spp	-	α	+	+	+
Pseudomonas aeruginosa	-	α	+	+	+
Streptococcus pyogenes	-	α	+	+	+
Shigella dysenteriae	-	α	+	+	+

Key: α= MIC value; + = Growth; - = No growth

Table 4: Minimum Bactericidal Concentration (MBC) of methanol root bark extract of *Boswellia dalzielii*

Microorganism	Concentration of Extract (mg/ml)				
	60	**30**	**15**	**7.5**	**3.25**
Salmonella typhi	-	-	B	+	+
Klebsiella spp	-	β	+	+	+
Pseudomonas aeruginosa	-	β	+	+	+
Streptococcus pyogenes	-	β	+	+	+
Shigella dysenteriae	-	β	+	+	+

Key: β= MBC value; +=turbid/bacterial growth; - =No turbid/no bacterial growth

The result of the phytochemical screening of the methanol extract of the root bark of *B.dalzielii* revealed the presence of carbohydrates, tannins, cardiac glycosides, glycosides, terpenoids, saponins, flavonoids, alkaloids and phenols in the extract. This study supports the reports of Nwinyi *et al*. (2004), Mamza *et al*. (2017; 2021) who revealed that *B. dalzielii* has significant antimicrobial activity.

The methanol extract contained terpenes which are reported to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties (Wagner and Elmadfa, 2003). Also, flavonoids are substances known to be synthesized by plants in response to microbial infections and have been reported to exhibit antimicrobial properties against wide variety of microorganisms. Biologically, the activity of flavonoidal compounds is probably due to their ability to complex with extracellular and soluble proteins and also to complex with bacterial cell walls (Cowan, 1999). Furthermore, steroids have been demonstrated to possess some antibacterial activity especially associated with membrane lipids and causes leakages from liposomes which eventually destroys/kill the microorganism (Rangari, 2012).

The MIC and MBC results gave values of 7.5 and 15 respectively were the lowest against *S. typhi*. The extract has also shown a remarkable bacteriostatic and bactericidal effect on Gram +ve and Gram -ve bacteria with MIC/MBC *against S. aureus, S. pyogenes, K. pneumonia* and *Shigella dysenteriae*. Abdulmumin *et al*. (2019), suggested that low values of MIC and MBC is indicative that of the drug material being potent. Therefore, the efficacy of the methanol extract extract has confirmed that the plant possesses phytochemicals of biological importance which can be used as natural antibiotics agents against the resistance pathogens.

CONCLUSION

This study has revealed that the methanol root bark extract of *B. dalzielii* contains some phytochemicals of biological significance. The results of the *in vitro* antimicrobial studies has scientifically proven the potency of the plant against resistant microbes especially *S. typhi, S. aureus, P. aeruginosa K. pneumonia and S. dysenteriae* and has justified the folklore use of this plant for the treatment of ailments such as fever.

REFERENCES

Abdulmumin, A. R., Kassim, Z, J., Muhammad, I. L., Mohammed, S. B. and Ya'aba, Y. (2019). Antimicrobial potency and bioactive ingredients of different extracts of *Prosopis africana* against some selected human pathogenic microbes: An *in-vitro* Approach. *Journal of Complementary and Alternative Medicine Research*. 8(1): 1-9.

Burkill, H. M. (1985). *Useful Plants of West Tropical Africa.* Volume one, Royal Botanical Gardens Kew. 300p.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review*. 12(4):564-82.

Danlami, U., Daniel, G. J., David, B. M. and Galadanchi, K. M. (2015). Phytochemical, nutritional and antimicrobial screening of hexane, ethyl acetate and ethanolic extracts of *Boswellia dalzielii* leaves and bark. *African Journal Bioscience and Bioengineering*. 3(5): 76-79.

Ethuk, E.U., Agaie, B.M., Onyeyili, P.A. and Ottah, C.U. (2006). Toxicological studies of aqueous stem bark extract of *Boswellia dalzielii* in albino rats. *Ind. J. Pharmacol.*, 38:359-360.

Evans, W.C. (2009). *Trease and Evans Pharmacognosy*. 16th Edition. Saunders Publishers, London. pp. 42–229.

Mamza, U. T., Sodipo, O. A., Abdulrahman, F. I. and Khan, I. Z. (2017). Proximate and elemental composition of methanolic stem bark extract *of Boswellia dalzielii* (frankincense tree: Burseracea). *International Journal. Science Research.* 6(10), 751-757.

Mamza, U.T., Yakubu, J., Chiroma, M., Balami, V.M., Moses, S., Sodipo, O.A., Abdulrahman, F.I., Alemika, T.E. and Khan, I.Z. (2021). Phytochemical evaluation and *in-vitro* antibacterial properties of the methanolic leaf extract of *Boswellia dalzielii* Hutch. (Burseraceae). *Bulletin Pure Applied Science-Chemistry.* 40C (2): 48-56.

Masoko, P., Picard, J. and Eloff, J.N. (2005). Antifungal activities of six South African Terminalia species (Combretaceae).

Journal of Ethnopharmacology. 99(2):301-308.

Nwinyi, F. C., Binda, L., Ajoku, G. A., Anaigu, S.O. and Gamaniel, K. S. (2004). Evaluation of aqueous extract of *Boswellia dalzielli* stem bark for antimicrobial activities and gastrointestinal effects. *African Journal Biotechnology*. 3: 284-288.

Rangari, V. D. (2012). Pharmacognosy and Phytochemistry. Career Publications vol. 1 3rd. Maharashtra, India. p55.

Shinkafi, T. S. Bello, L., Hassan, S. W. and Ali, S. (2015). An ethnobotanical survey of antidiabetic plants used by Hausa–Fulani tribes in Sokoto, Northwest Nigeria. *Journal Ethnopharmacology*. 172: 91–99.

Sofowora, A. (2008). Medicinal Plants and Traditional Medicine in Africa. 3rd ed. Ibadan, Nigeria: Spectrum Books Limited.

Usman, H., Abdulrahman, F.I. and Ladan, A.A. (2007). Phytochemical and antimicrobial evaluation of *Tribulus terrestris L. (Zygophylaceae)* growing in Nigeria. *Research Journal Biological Science.* 2(3): 244-248.

Vollekova, A., Kostalova, D. and Sochorova, R. (2001). Isoquinoliniia microbioline alkaloids from *Mahania aquifolium* stem bark is active against *Mulassezia spp. Folia*, 46: 107-111.

Wagner, K.H. and Elmadfa, I. (2003). Biological relevance of terpenoids Overview focusing on mono-, di- and tetraterpenes. *Annal Nutrition and Metabolism*. 47(3-4):95-106. 25.