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Phytochemical Screening and Antibacterial Activity of *Boswellia dalzielii* Stem Bark Extract Against Clinical Isolates of *Staphylococcus aureus, Escherichia coli, Salmonella Spp* and *Pseudomonas aeruginosa*

Aliyu, M.Y., Shariff, H.G , Adam, A.J.

Department of Applied Biology, College of Science and technology, Kaduna polytechnic, Kaduna

ABSTRACT: In recent times attention has been directed to medicinal research to substantiate the claims of cures made by traditional healers, thereby providing scientific basis for their efficacy. This study was carried out to determine the bioactive components and antibacterial activity of *Boswellia dalzielii* stem bark extract against clinical isolates of *Staphylococcus aureus, Escherichia coli, Salmonella species, and P. aeruginosa* respectively. The aqueous stem bark extract revealed the presence of tannins, flavonoids, alkaloids, steroids and cardiac glycosides. The methanol stem bark extract revealed the presence of saponins, tannins, flavonoids, steroids and cardiac glycosides. The antibacterial activity of the leaf extract was assayed using agar well diffusion method. The aqueous extract showed antibacterial activity against the test bacterial isolates with the highest activity against *Salmonella species* with mean zone of inhibition of (27. 7 ± 0.3 at 100 mg/ml), followed by *S. aureus* (25.3 ± 0.3 at 100mg/ml) and the least activity was observed in *S. aureus* with the mean zone of inhibition of (12. 3 ± 0.3 at 12.5 mg/ml). The methanol extract showed antibacterial activity against the test bacterial isolates with the highest activity against *E. coli* at (18.3± 0.3 at 100mg/ml), followed by *P.aeruginosa* (14.6±0.3 at 100mg/ml) and the least activity was observed in *Salmonella spp* with the mean zone of (9.6± 0.5mg/ml). The zones of inhibition were compared with a commercial antibiotic, ciprofloxacin. The minimum inhibitory concentration of the extract against the test isolates *P. aeruginosa*, *E. coli, Salmonella spp and* S. *aureus* were found at the concentrations of 12.5mg/ml 25mg/ml. 50mg/ml, 100mg/ml and respectively, while the minimum bactericidal concentration for *P. aeruginosa*, *E. coli, Salmonella spp and* S. *aureus* were found at the concentrations of 25mg/ml, 50 mg/ml 100mg/ml and100mg/ml respectively. The results of this study therefore indicates that the plants stem bark contain potent phytochemicals inhibitory to bacteria, this could pave a way of obtaining new and effective herbal medicine to treat bacterial illnesses.

KEY WORDS: ***phytochemicals, antibacterial activity, Boswellia dalzielii, stem bark, isolates.***

I. INTRODUCTION

Plants used in traditional medicine may constitute an important source of new biologically active compounds. It is estimated that there are about 2,500 000 species of higher plants and the majority of these plants have not been studied for their pharmacological activities. The world health organization reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies which involve the use of plants extracts (WHO, 2010).

During the last decades, the limit of microbial diseases and infections has been exceeded drastically (Wael *et al.*, 2015). A persistent problem in antimicrobial chemotherapy is the increasing occurrence of resistance to antibiotics, which leads to the recalcitrance of antimicrobial treatment (Raubilu *et al.*, 2019). The continuing usage of antibiotics and consequent antibiotic selection pressure is thought to be the most crucial factor contributing to the appearance of several kinds of resistant microbes (Bajpal *et al.*, 2014).

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Various specific plants have continued to be an important therapeutic aid for alleviating the ailments of human kind. Therefore, novel antimicrobial agents from different biological sources are continuously sought. Research conducted on medicinal plants have served the dual purposes of bringing up new therapeutic agents and providing useful leads for studies directed towards the synthesis of drugs on the basis of the chemical structures of the natural products with modern pharmaceutical industries still relying to some extent on the bioactive principle, obtained from plants (Olukemi *et al.,* 2005). *Boswellia dalzielii* (family Burseraceae), commonly known as frankincense tree; abounds in the Savannah regions of West Africa. It has been employed in Ethno medicine with various degree of success. Its parts have also been employed and found to contain numerous phytochemicals such as tannins, glycosides, flavanoids, alkaloids, anthraqunones, saponin and saponins glycosides, as reported by (Raubilu *et al.,* 2019). Its aqueous stem bark extracts is used as anti-diarrhea (Etuk *et al.,* 2006) antispasmodic (Hassan *et al.,* 2009) and anti-ulcer (Nwinyi *et al.,* 2004).

In addition, it has been used in folk medicine as antiseptic, anti-arthritic, wound healing, anti-malaria, anti diarhoea, anti-inflammatory, anti bacteria, anti fungal, ant-hepatitis, antidotes to arrow poison and for the treatment of rheumatism, leprosy, gastro-intestinal troubles (Josh *et al.,* 2013). The stem bark is used to treat rheumatism, septic sores, veneral diseases and gastrointestinal ailments (Olukemi *et al.,* 2005). The leaves are used in large quantity to make a wash of fever and rheumatism while it is also taken internally for gastrointestinal troubles (Burkill, 1985). Report have also shown that the plant has been used for the treatment of dental problems, swilling, bronchitis, cough, gastric disorder, asthmatic attach, pulmonary diseases and skin ailments, among others (Baoua *et al.,* 1976).This study was aimed at evaluating the phytochemical components and antibacterial activity of *Boswellia dalzeilii* aqueous and methanol stem bark extracts against *Staphylococcus aureus*, *Salmonella species*, *Pseudomonas aeruginosa* and *Escherichia coli.*

II. MATERIALS AND METHODS

Sample Collection Identification and Authentication of the Plants.

The stem bark of *Boswellia dalzielii* was collected from Jigawa state. The identification and authentication of the plant materials was done at herbarium of the department of plant Sciences, Bayero University Kano with voucher number BUKHAN 0381, voucher specimens were deposited there for future reference.

Extraction of plant materials

The parts collected were washed thoroughly with distilled water and air-dried in a shade for two weeks, it was then cut into pieces and grinded to powder using a sterile pestle and mortar under laboratory condition. The powder was then kept in air tight container for future use. The procedure was carried out according to the method (Sofowora, 2003).
Extraction of plant material was carried out using maceration method according to (Sofowora, 2003). One hundred (100g) of the grinded samples each were extracted by successive soaking for 4 days with intermittent shaking using 500ml of methanol and 500ml of distilled water separately in a sterile conical flask. The extract were filtered using Whatman filter paper to remove all forms of residue and the extracts were evaporated to dryness using rotary evaporator and stored at 4^{o}C for further analysis (Akinnibosun, *et al.,* 2009).

Phytochemical screening of *Boswellia dalzelli* extracts

The crude extracts of both aqueous and methanol were subjected to phytochemical screening for the presence of the following bioactive components Saponins, Alkaloids, Tannins, Steroids, Flavonoids, Glycosides, Carbohydrates, Anthaquinone (Sofowora, 1984).

Preparation of McFarland standard and standardization of inoculum

Nutrient broth was prepared according to manufactures specifications. The bacterial isolates were tested for sterility on nutrient Agar then re-grown in Nutrient broth at 37^{o}C for 24hrs. McFarland's standard method was adopted to standardize the organisms to1×10^{8} cfu/ml using Genesy's 20 spectrophotometer. 99.5ml of % $Bacl_2$ was added to 0.5ml of 1% H_2SO_4 in order to obtain 100ml of $BaSO_4$ which corresponded to 0.5×10^{8} cfu/ml McFarland standards for the bacterial test organisms at 625nm optical density. The overnight cultures of the test organisms were prepared and matched with the McFarland standard. Dilution with sterile nutrient broth was made until the optical density matched that of McFarland's standard (Cheesbrough, 2002)

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Antibacterial Assay

The agar well diffusion method of Kirby-Bauer as described by (Cowan, 2015) was adopted to test for the antimicrobial activity of the extracts on the test organisms. 15cm sterile disposable media plates of nutrient agar well prepared for the bacterial according to the manufacturers specifications. These plates were then separately flooded with diluted standardized overnight cultures and then drained to remove excess. Four wells of 6mm diameter were made in each plate with a central well for the control using 6mm sterile cork borer. The wells were filled with 0.1ml diluted concentrations (100mg /ml, 50mg /ml, 25mg/ml and 12.5mg/ml) of extracts with the aid of sterile pipettes per well. While 20mg/ml of the standard antibiotics ciprofloxacin were used as positive control. Sterile distilled water was used as negative control on a separate plate. Diameters of zones of inhibition were measured using millimeter ruler after incubating the plates at 37^{o}C for 24hrs (Nas and Ali, 2017). The plates were replicated in triplicates and the means of the zones of inhibition for each organism at each concentration of the extracts were calculated and recorded

.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of methanol and aqueous extracts of the *Boswellia dalzielii* were determined by the broth dilution method using serially diluted plant extracts according to the national committee for clinical and laboratory standard (NCCLS, 2007) Protocol as described by Doughari *et al.*, (2007). About 0.5ml of varying concentration of the extracts (100mg/ml, and 50mg/ml, 25mg/ml, 12.5/ml, 6.25mg/ml) were added in test tubes, 2ml of nutrient broth was added and then a loopful of the test organisms previously diluted to 0.5 Mcfarland turbidity standards was introduced. The procedure was repeated on the test organisms, using the standard antibiotics (ciprofloxacin). A tube containing nutrient broth only was use as control. The culture tubes were then examined for bacterial growth by observing the turbidity as described by El- Mahmood *et al.* (2018).

Determination of Minimum Bactericidal Concentration

The MBCs of the extracts were determined using the method described by Adegboye *et al*., (2008). Samples were taken from the tube with no visible growth with MIC assay and Sub cultured on to fresh prepared nutrient agar medium and later incubated at 37^{o}C for 24hrs. The MBCs were taken as the lowest concentration of the extract that did not allow any bacterial growth on the surface of the agar plates as described by Chessbrough, (2000)

III. RESULTS

The result of the physical characteristics of methanol stem bark extract of *Boswellia dalzielii* was observed to be brownish-green with a percentage yield of 12.8% while that of aqueous extract appeared brown with percentage yield of 27.6% (table 1).

Table 2 shows the result of phytochemical screening of *Boswellia dalzielii* stem bark extracts which revealed the presence of saponins, alkaloids, tannins, flavonoids, steroids and cardiac glycosides, while methanol extract shows the present of saponins, tannins, flavonoids, steroid and cardiac glycosides. The antibacterial activity of the stem bark extract is presented on table 3. All the concentrations of the extracts showed activity against *E. coli, Salmonella* spp, *S. aureus and Pseudomonas aeruginosa*. The organisms were susceptible at different concentrations of the extract. As such the activity was found to be dependent on the concentration of the extracts.

The minimum inhibitory concentrations, (MIC) of the aqueous and methanol stem bark extracts of *Boswellia dalzielii* were found to be inhibitory to *E. coli* at 25mg/ml, for *Salmonella* spp MIC was found at 25mg/ml and 6.5mg/ml aqueous and methanol stem bark extract respectively. Similarly MIC of aqueous and methanol extract against *S. aureus* was 50mg/ml and 12.5mg/ml respectively, *P. aeruginosa* revealed 12.5 mg/ml and 25mg/ml MIC for both aqueous and methanol extracts. The minimum bactericidal concentration (MBC) of the stem bark of *Boswellia dalzielii* aqueous and methanol extract against all the test isolates was found ranging from 12.5mg/ml to 100mg/ml.

Table 1: percentage yield of Aqueous and Methanol stem bark extract of *Boswellia dalzielii*

Crude extract	Initial sample weight (g)	Percentage yield %	Appearance
Methanol	100	12.8	Brownish green
Aqueous	100	27.6	Brown

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Table 2: Phytochemical Constituents of Methanol and Aqueous Extracts of *Boswellia dalzielii* Stem bark.

Phytochemical constituent	Methanol extract	Aqueous extract
Saponins	+	-
Alkaloids	-	+
Tannins	+	+
Anthraquinone	-	-
Flavonoids	+	+
Steroids	+	+
Cardiac glycosides	+	+

Key: + detected, - not detected

Table 3: Antibacterial activity of aqueous and methanol stem bark extracts of *Boswellia dalzielii* against the bacterial isolates

Test organisms	Concentrations (mg/ml) (Methanol extract) Diameter zone of inhibition(mm)					Concentrations (mg/ml) (Aqueous extract)				
	100	50	25	12.5	Control (Ciprofloxacin)	100	50	25	12.5	Control (Ciprofloxacin)
E. coli	20.7 ±0.5	17.7 ±0.3	16.3 ±0.3	14.7 ±0.3	24.2±0.3	18.3 ±0.3	16.3 ±0.3	15.3 ±0.3	13.3 ±0.3	25.0±0.4
S. aureus	25.3 ±0.3	15.7 ±0.3	16.7 ±0.3	12.3 ±0.3	32.7±0.5	17.0 ±0.4	14.3 ±0.3	12.3 ±0.5	10.4 ±0.4	24.7±0.5
S. typhi	27.7 ±0.3	21.7 ±0.4	16.7 ±0.5	12.5 ±0.3	38.0±0.4	15.7 ±0.3	13.3 ±0.3	11.9 ±0.4	9.6 ±0.5	38.7±0.3
P. aerugi nosa	21.8 ±0.3	18.7 ±0.5	16.4 ±.04	13.4 ±0.2	32.0±0.2	14.6 ±0.2	12.3 ±0.3	14.3 ±0.4	15.0 ±0.4	22.7±0.5

Diameter zone ofnhibition ± standard deviation

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Table 4: Minimum inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of the extracts against the test isolates

Test Isolates	Concentration (mg/ml)			
	Aqueous extract		Methanol extract	
	MIC(mg/ml)	MBC(mg/ml)	MIC(mg/ml)	MBC(mg/ml)
Escherichia coli	25	50	25	50
Salmonella spp	25	100	6.25	12.5
Staphylococcus aureus	50	100	12.5	25
Pseudomonas aeruginosa	12.5	25	25	100

IV. DISCUSSION

Results from the phytochemical analysis reveals the presence of steroids, alkaloids, glycosides flavonoides, saponins and tannins .The result of this study is in line with the findings of Prakesh *et al.* (2015) and Nas and Ali, (2017), who detected the presence of similar components in both aqueous and methanol extracts of *Boswellia dalzielii*. Various studies have shown that plants that are rich in alkaloids and tannin compounds possess antimicrobial activity usually against a wide range of microorganisms (Giwa *et al.*, 2012). There has been an earlier report on the antibacterial activity of *B.dalzielii* stem bark and oil from its leaves (Nwinyi, 2004; Olukemi *et al.*, 2002). The plant could be an important precursor for the development of an antimicrobial.

The antibacterial activity reveals that all the test isolates were susceptible to the plants extracts as evidenced by differences in diameter zones of inhibition. The susceptibility of all the isolates is comparable at all the concentration suggesting that *B. dalzeilli* may be active against both Gram positive and Gram negative bacteria. The extracts are more active at high concentration and less active at low concentration. This suggests that the activity of the extracts is dose dependents with the highest activity observed at 100mg/ml and the lowest at 6.25mg/ml.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration of the extract showed that methanol extracts had the lowest MIC and MBC values in the ranges of (6.25-25mg/ml) and (12.5 – 50 mg/ml) respectively while aqueous extract has MIC and MBC ranges of (12.5 – 50 mg/ml) and 25-100mg/ml respectively. The result showed that the crude extract of the plant have strong activity against the isolates used in this study. Thus, the extracts have spectrum of activity and this is inconformity with the finding of Ntiejumokwu and Alemika (1991) who reported that the extracts of *B. dalzielii* have a broad spectrum of activity against both gram positive and gram negative bacteria. The fact that the extracts are active against some members of Enrerobacteriaceae confirmed the ethno botanical usage of the plant in treating gastroenteritis particularly those caused by the organisms. MICs of the extracts were lower than the MBCs, suggesting that the extracts were bacteriostatic at lower concentration and bactericidal at higher concentrations. This support the report of Aliyu *et al.* (2008), who evaluated the antibacterial activity of 12 medicinal plants used in Northern Nigerian traditional medicine against 25 hospital isolates of methicillin resistant *Staphylococcus aureus* (MRSA). The different values obtained from MBC of stem bark extracts on *E. coli* and *S. aureus* confirm that both extracts have varying phytochemical properties and hence exhibit different inhibitory effects and bactericidal effect on the test organisms but less compared to standard antibiotics used.

V. CONCLUSIONS

In this study, methanol was found to be the solvent of choice for extracting bioactive phytochemical from leaves and stem bark of B. dalzielii. The active phytochemical components found in this study include alkaloid, saponin, tannin, flavonoids, and glycosides. The study confirmed that the stem bark extract of *B. dalzielii* exhibited significant antibacterial activities against clinical isolates of *Staphylococcus aureus, Salmonella* spp *Escherichia coli* and *Pseudomonas aeruginosa* at varied concentrations, supporting the traditional usage of the plant parts in treating many diseases involving the microbes. Based on the results obtained it is strongly recommended that more attention should be given to medicinal plant as a cheaper and more easily assessable source of treatment for mild cases of ailments and more research should be carried out to determine the toxicity and antifungal activity of the plant extracts.

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