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ANTIMICROBIAL ACTIVITY OF ETHANOL AND AQUEOUS EXTRACTS OF BOSWELLIA DALZIELII AGAINST ENTEROPATHOGENIC ESCHERICHIA COLI AND SHIGELLA DYSENTERIAE

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ABSTRACT

Ethanol and aqueous extracts of stem bark of Boswellia dalzielii was used to determine the phytochemical and antibacterial activity against enteropathogenic E. coli and S. dysenteriae. The extraction was carried out using a standard method. The phytochemical screening was carried out through standard procedures and the extracts were further tested for antimicrobial activity using agar well diffusion method. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were also determined. Ethanol extract has the highest percentage yield of 22.26, the phytochemical tests revealed the presence of saponins, tannins, flavonoids, terpenoid, glycosides, alkaloids, triterpenes, phenols and proteins. Antimicrobial activity shows that ethanol extract has the highest mean zone of inhibition of 17.67mm and 17. 00mm at the highest concentration (250mg/ml) against S. dysenteriae and E. coli respectively, both test organisms were resistant to all concentrations of aqueous extract except at the highest concentration. MIC of aqueous extract was 250mg/ml and MBC of 500mg/ml was shown on both the test organisms. The study shows that Boswellia dalzielii stem bark ethanol and aqueous extract contain bioactive components which are responsible for its antimicrobial activities and may have the potential for the development of plant source drugs against diarrhea causing E. coli and S. dysenteriae.

Keywords: Boswellia dalzielii, phytochemical, Escherichia coli, Shigella dysenteriae, Antimicrobial.

INTRODUCTION

Ethnomedicine refers to the study of traditional medical practice which is concerned with the cultural interpretation of health, diseases and illness and also addresses the healthcare seeking process and healing practices (Krippner, 2003). Today about 80% of the world's population rely predominantly on plants and plant extracts for healthcare (Setzer *et al*, 2006).

Boswellia dalzielii Hutch belong to the family Burseraceae, and commonly known as the frankincense tree that grows up to 13m high, the plant is found mainly in the Savannah region of West Africa. The tree has a characteristic pale papery bark that is peeling and ragged. The Hausa names include "hanuu", "Ararrabi" and "Basamu", the plant has several medicinal uses, decoction of the stem bark is used in North-Central part of Nigeria in the treatment of mental illness (Ibrahim *et al*., 2007). The stem bark has been found to contain chemical constituents including phenolic compounds such as protocatechuic acid, gallic acid and ethylgallate as well as a diterpenoid - incensole and a triterpenoid- 3-O-acetyl-11-keto-b-boswellic acid (Olukemi *et al*., 2005). Nazifi *et al.* (2007) reported the presence of cardiac glycosides, flavonoids, saponins and tannins through thin layer chromatographic analysis of methanol extract of *B. dalzielii*, their results suggest central nervous system depressant action of stem bark extract of *Boswellia dalzielii* which might have contributed to its application in ethnomedicine for the treatment of mental disorders. The work of Nwinyi. (2004) revealed that the aqueous extract of *B. dalzielii* (2 mg/ml) did not inhibit the growth of *Bacillus subtilis*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aerugniosa*, *Proteus mirabilis* and *Escherichia coli* tested. But the plant has shown that the aqueous extract of the stem bark produced some anti-ulcer activity.

Enteropathogenic Bacteria are the principal causes of high infant and child (under 5 yrs) mortality as well as morbidity in all age groups, especially in aged and immune compromised patients, in developing countries. Thus clinical management of these infections can be complicated and unsuccessful (Shakti and Rabindra, 2015). The presence of enterobacteria in foodstuffs and water is a common cause of diarrhea and dysentery among the infant population (Viera *et al*., 2001). Pathogenic *E. coli* and *Shigella* spp. are ubiquitous Gram-negative rod shaped bacilli largely associated with mammalian or avian hosts, classified in the genus *Shigella*, within the family Enterobacteriaceae (The *et al*., 2016). Diarrhea diseases constitute a major cause of morbidity in children. These diseases account for approximately 5–10 million deaths each year in Asia, Africa and Latin America, and are the major cause of death among 15%–20% of children under 5 years old (Lee, 2000). This paper aimed at testing the efficacy of ethanol and aqueous extracts of *Boswellia dalzielii* used traditionally in Kano state, northern Nigeria, for the treatment of diarrhea in children less than 5 years.

MATERIALS AND METHODS

Collection of plant materials

The stem bark of *Boswellia dalzielii* was freshly collected from Fagwalawa area of Dambatta Local Government Area in Kano state in April 2016. The plant was identified and authenticated at the herbarium of the Department of Plant Biology, Bayero University Kano. Voucher number BUKHAN 0362 was given; Specimen was deposited at the herbarium of the Department.

Extraction of the plant materials

Stem bark of *B. dalzielii* was air dried and crushed into powdered form using a pestle and mortar, and the powdered sample was extracted following the method of Gupta *et al.*(1996). One hundred grams (100g) of the dried powder was weighed into separate glass containers and percolated with 500ml each of the (2) different solvents, the two organic solvents chosen were ethanol and water. The percolates were left for five days with vigorous hand shaking at intervals, the mixtures were filtered through Whattman no. 1 filter paper, the filtrates were concentrated to dryness at 40^{o} -45^{o} C using water bath, and the extracts were subsequently transferred into sterile glass containers, weighed and stored in a refrigerator for further use. The extracts were viewed by eyes to determine the physical appearance and colour, smelled to determine the odor and touched by hand to determine the texture.

Phytochemical screening of plant materials

All the plant extracts were analyzed for the presence of the following phytochemical groups; saponin, tannin, flavonoid, glycosides, alkaloid, carbohydrate, triterpenes, phenols and protein, according to standard methods described by Herbone, 1998, Evans, 1998 and Poongothai *et al.,* 2011.

Collection, identification and biochemical characterization of test organisms

Clinical isolates of enteropathogenic *Escherichia coli* (EPEC) and *Shigella dysenteriae* were obtained from Microbiology Department of Murtala Muhammed specialist Hospital. The test organisms were collected from under five years children, and the isolates were identified in the microbiology lab of Bayero University, Kano using biochemical characterization (Cheesebrough, 2009). The isolates were then maintained on nutrient agar slant and stored at 4^{0}C in a refrigerator. Biochemical confirmation of the test organisms was achieved using Indole, Methyl-red, Voges- Proskauer and Simon's citrate (IMVIC) test, as described by Cheesebrough. (2009). the isolates were sub cultured on nutrient agar plates before the tests were carried out.

Standardization of the test organisms

The standardization of the bacteria was done using a method described by Clinical Laboratory Science Institute (CLSI). 2012, as follows; a loop full of fresh colony from an overnight subculture of the test bacteria was inoculated into a test tube containing sterilized normal saline using a sterile wire loop, the test organisms were suspended until the turbidity of the suspension matches the turbidity of 0.5 McFarland standard, equivalent to 1.0×10^{8} cfu/ml population for bacterial isolates.

Preliminary antibiotic susceptibility test

The susceptibility of the two bacterial isolates against eight antimicrobial agents, which includes (ceftazidime, cefuroxime, gentamicin, cexime, ofloxacin, augmentin, nitrofurantion and ciprofloxacin), was determined using disc diffusion Kirby Bauer's method, and as recommended by the guideline of Clinical and Laboratory Standard Institute (CLSI).(2014).

Preparation of extracts stock concentration for antimicrobial screening

Four (4) different concentrations of each extract where prepared for antimicrobial susceptibility test through serial dilution, 0.5g of the plant extract was dissolved in 2ml of dimethyl sulphuroxide (DMSO) to arrive at 500,000ug/ml (500mg/ml), this served as stock solution, from the stock solution through serial dilution the following concentrations were obtained; 250,000ug/ml (250mg/ml), 125,000ug/ml (125mg/ml), 62,500ug/ml (62.5mg/ml) and 31,250ug/ml (31.25mg/ml).

Antibacterial sensitivity test

The antibacterial susceptibility assay was conducted using agar well diffusion method as described by Rath *et al*. (2013), using Mueller Hinton agar and ciprofloxacin 250mg/ml as positive control. The antibacterial activities of the extracts were determined after 24 hrs by measurement of diameter of zones of inhibition produced by the extracts against the test organisms and the results were recorded in millimeters (mm).

Determination of minimum inhibitory concentration and minimum bactericidal concentration

Minimum inhibitory concentration (MIC) of the two plant extracts was determined using serial double dilution method using Dimethyl-Sulfoxide (DMSO) to arrive at concentrations of 15.63mg/ml, 31.25mg/ml, 62.5mg/ml,125mg/ml,250mg/ml and 500mg/ml (CSLI, 2012). Depending on the least concentration of the extract that inhibited the growth of the test organism from the agar well diffusion bioassay, four concentrations were selected to determine the MIC of the extract. A series of 6 test tubes were arranged on a test tube rack for each extract, in the first test tube 4ml of normal saline was introduced and in the remaining 5 test tubes 2ml of the normal saline was introduced, following serial doubling dilution method, all the 6 test tubes contain 2ml normal saline and the excess 2ml was discarded, and each contained varying serial doubling concentration of the extract. Thereafter, 0.1ml of the standardized test organism was introduced in the first four test tubes, in the last two test tubes, one contained test organism and the growth medium without extract and served as positive control while the last test tube contained extract and the growth medium without inoculums and served as negative control. The test tubes were incubated at 40^{o}C for 18 – 24hrs and then examined; the concentration of the extract that inhibits the growth of the bacteria, which is the concentration with no observed turbidity when compared with the control tubes, was taken as the minimum inhibitory concentration.

Minimum bactericidal concentration (MBC) was determined by sub-culturing the test dilution samples from the MIC tubes that

showed no visible growth on sterile Mueller Hinton agar plates and then incubated for further 18 – 24 hrs. The dilution that yielded no single bacterial colony on the solid MHA was taken as the minimum bactericidal concentration (MBC).

Data analysis

Data was presented as means ± standard error (SEM), and the significant difference between different groups was tested using analysis of variance (ANOVA), the treatment means were compared with Duncan's new multiple range test (DNMRT) using SAS system software. The significance was determined at the level of $P \leq 0.05$.

RESULTS

Table 1 shows the percentage yield of the two different plant extracts, water showed to have higher percentage yield of 22.26% whereas ethanol had 3.24% of the residue recovered.

Table 1: **Percentage yield of the two different plant extracts**

Plant	Solvent	Original weight percolated (g)	Weight of residue recovered	% yield
B. dalzielii	Water	100	22.26	22.26
	Ethanol	100	3.24	3.24

The physical properties of the plant extracts used was shown in table 2, both the different extracts have the same creamy and woody odour, while the colour appeared to be red for water extract and greenish brown for ethanol extract, the texture was soft and dry for the water and ethanol extracts respectively.

Table 2: Physical properties of plant extracts

Extract	Appearance	Colour	texture	Odour
Water	Creamy	Red	Soft	Woody
Ethanol	Creamy	greenish brown	Dry	Woody

The phytochemical properties of *Boswellia dalzielii* extracts as shown in table 3, revealed the presence of all the phytochemical tested except carbohydrates and triterpenes in ethanol extract while the aqueous extract shows the presence of saponin, tannin, triterpenes, phenols and protein.

Table 3: Phytochemical properties of *Boswellia dalzielii* extracts

	Solvents	
Phytochemicals	**D/Water**	**Ethanol**
Saponin	+	+
Tannin	+	+
Flavonoid	-	+
Terpenoid	-	+
Glycoside	-	+
Alkaloid	-	+
Carbohydrate	-	-
Triterpenes	+	-
Phenols	+	+
Protein	+	+

Key: + = present, - = absent

Table 4 shows the result of biochemical confirmatory test of the two bacterial isolates, *E. coli* tested positive to indole test while *S. dysenteriae* tested negative, both test organisms were positive for methyl red test and all negative for voges- poskauer and simon's citrate tests.

Table 4: Biochemical confirmatory test (IMVIC) on two bacterial isolates

	Test bacteria	
Test type	***E. coli***	***S. dysenteriae***
Indole	+	–
Methyl Red	+	+
Voges-Proskauer	–	–
Simon's citrate	–	–

Key: + = positive, - = negative

Table 5 shows the effect of the extracts concentrations on the growth of the different bacterial species used in the study, 14.97mm was the highest mean zone of inhibition of 250mg/ml on *E. coli* and it was significantly different from the same concentration on *S. dysenteriae* (13.28mm) and all other concentrations. Concentration of 125mg/ml on *E. coli* (11.75mm) was not significantly different from 250mg/ml on *S. dysenteriae* (13.28mm), there was also no significant difference between the growths of the two organisms at 62.5mg/ml, but at 31.25mg/ml the difference in growth of the bacterial isolates was significantly different.

Table 5: Effects of plant extract concentrations on the growth of the clinical isolates

	Zones of inhibition	
Concentrations (mg/ml)	***E. coli***	***S. dysenteriae***
	Mean ± SE	Mean ± SE
31.25	5.75 ± 0.89^{e}	4.14 ± 0.81^{f}
62.5	8.33 ± 0.90^{d}	7.67 ± 0.76^{d}
125	$11,75 \pm 0.89^{bc}$	10.36 ± 0.77^{c}
250	14.97 ± 0.70^{a}	13.28 ± 0.71^{b}

Keys: Means with the same superscript indicate no significant difference.

As shown in table 6, effect of different concentrations of ethanol extract at 250mg/ml has the highest mean zone of inhibition of 17.00 ± 2.00 and 17.67 ± 1.67 against *E. coli* and *S. dysenteriae* respectively which was not significantly different between the two organisms, but significantly different between all the remaining concentrations, however, 11.00 ± 1.00 against *S. dysenteriae* was the least mean zone of inhibition recorded at the least concentration used (31.25mg/ml). In general increase of zone of inhibition was shown with the increase in concentration of the plant extract.

Table 6: Effect of different concentrations of ethanol extracts of the plant specie on the growth of the test organisms

Plant	Solvent	Zones of inhibition (mm) Concentration(mg/ml)	*E. coli*	*S. dysenteriae*
			Mean ± SE	**Mean ± SE**
B.dalzielii	Ethanol	31.25	12.33 ± 3.39^{cd}	11.00 ± 1.00^{d}
		62.5	13.33 ± 3.34^{c}	13.00 ± 1.00^{c}
		125	15.00 ± 2.52^{b}	15.33 ± 1.34^{b}
		250	17.00 ± 2.00^{a}	17.67 ± 1.67^{a}

Key: Means with the same superscript indicate no significant difference.

Table 7 shows the effect of different concentrations of the water extracts of *B. dalzielii* on the growth of the test organisms, there was no zone of inhibition in all the concentrations used against both test bacteria except at the highest concentration (250mg/ml), however, there was significant difference of zone of inhibition between the two organisms at the level of concentration where they show susceptibility.

Table 7: Effect of different concentrations of aqueous extracts of the plant specie on the growth of the test organisms

Plant	Solvent	Zone of inhibition (mm) Concentration(mg/ml)	*E. coli*	*S. dysenteriae*
			Mean ± SE	Mean ± SE
B.dalzielii	Aqueous	31.25	0.00 ± 0.00	0.00 ± 0.00
		62.5	0.00 ± 0.00	0.00 ± 0.00
		125	0.00 ± 0.00	0.00 ± 0.00
		250	8.00 ± 0.00a	5.33 ± 2.67^{b}

Key: Means with the same superscript indicate no significant difference.

The minimum inhibitory concentration and minimum bactericidal concentration of *B. dalzielii* stem bark water and ethanol extracts against the test organisms was shown in table 8, the MIC of aqueous extract was at 250 mg/ml and MBC at 500mg/ml on both the test organisms while in ethanol extract, MIC of 31.25mg/ml and 62.5mg/ml was recorded for *E. coli* and *S. dysenteriae* respectively with MBC of 250mg/ml for both the test organisms.

Table 8: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Boswellia dalzielii* stem bark extracts on two clinical isolates of bacteria

Extracts (mg/ml)					
		Aqueous		**Ethanol**	
S/N.	Test organisms	MIC	MBC	MIC	MBC
1.	*E. coli*	250	500	31.25	250
2.	*S.dysentereae*	250	500	62.5	250

DISCUSSION

From the results obtained, water is the best solvent of extraction in *B. dalzielii* stem bark as it gives the highest yield of extract; this is similar to the work of Usman. (2016), who reported water as the solvent with the highest yield of extracts used in the extraction of the leaves of *Citrus sinensis* and *Eucalyptus comaldulensis*. The physical properties of the plant's different solvents, revealed some variations in colour as ethanol has greenish brown colour while aqueous has red colour as well as dry and soft texture respectively. This corroborate with the report by Nazifi *et al.* (2017), that methanol extract of *B. dalzielii* was semi-solid in nature and possesses dark brown colour with honey-like smell. The result of phytochemical screening of crude ethanol and aqueous extract of the stem bark of *Boswellia dalzielii* revealed the presence of secondary metabolites such as alkaloid, tannins, saponins, flavonoids, glycosides, steroids, triterpenes, protein and phenols. These metabolites have been reported to possess antimicrobial activity against several pathogens (Hassan *et al*., 2004). Tannins have been reported to inhibit growth of microorganism by precipitating microbial protein and making nutritional proteins unavailable to them (Ogunleye and Ibitoye, 2003).

The result of antibacterial activities of stem bark of the ethanol and aqueous crude extracts of *B. dalzielii* against test isolates at various concentrations revealed all the isolates to be sensitive for all ethanol extract concentrations and non sensitive to all

water extract concentration except at the highest concentration used (250mg/ml).this agrees with the work of Nwinyi. (2004) who reported that the aqueous extract of *B. dalzielii* at 2mg/ml did not inhibit the growth of all the tested bacteria among which is *E. coli*. Zone of inhibition at the lowest concentration of 31.25mg/ml for ethanol was observed by *B. dalzielii* (12.33 and 11.00) mm against *E. coli* and *S. dysenteriae* respectively, Mosses et *al.* (2005) reported some degree of broad spectrum activity of *B. dalzielii* ethanol extract including *E. coli*. No zone of inhibition was observed for all the concentrations of aqueous extract except at 250mg/ml on both the test organisms. Inactivity of aqueous extract of *C. senegalensis* against *E. coli* was reported by Usman. (2007), this may be due to very low concentrations used (10ug/ml). *B. dalzielii* stem bark ethanol extract was able to inhibit the growth of *E. coli* at 31.25mg/ml and *S. dysenteriae* at 62.5mg/ml with MBC of 250mg/ml on both the test organisms, this finding is similar but not in agreement to that of Olukemi *et al.* (2005), who reported MIC of *B. dalzielii* ethanol extract on *E. coli* at 15mg/ml and MBC of 30mg/ml, this differences may be due to the concentration and the test organism used. The MIC of aqueous extract was 250 mg/ml and MBC of 500 mg/ml on both test organisms.

Generally differences in the concentration recorded differences in the diameter zone of inhibition on both the test organisms, lower zone of inhibition were recorded with lower concentration, as there is increase in the concentration there is increase in the diameter zone of inhibition.

CONCLUSION

In conclusion, stem bark of *Boswellia dalzielii* may have the potential for the production of drug for the treatment of diarrhea infections caused by enteropathogenic *Escherichia coli* and *Shigella dysenteriae* due to presence of bioactive constituents which are responsible for antimicrobial activity of the extracts. The result of this study also indicated that ethanol extract of stem bark of *Boswellia dalzielii* was more effective than the aqueous extract at the concentration used, showing that with more concentration there may be higher zone of inhibition. The study therefore proves the continuation of the use of stem bark of *Boswellia dalzielii* for the treatment of diarrhea and dysentery by indigenous people in traditional medicine. Toxicological studies of *Boswellia dalzielii* stem bark extracts to determine its toxicity level is recommended.

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