



Phytoconstituents, Antioxidants and Antimicrobials Activity of Leave, Stem Bark and Roots of *Boswellia dalzielii* on Selected Bacterial Isolates

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Abstract

Introduction; *Boswellia dalzielii* is a very unique medicinal plant that is part of the Burseraceae family. It has been regarded as a wonder plant in northern part of Nigeria and is called commonly as “ararrabi or hanu” by the hausa speaking communities or frankincense tree in English. All parts of the plant have been reported to have varying medicinal significance. The fresh bark is used to treat giddiness and palpitations **Aims;** The present study is carried out to evaluate the phytoconstituents, antioxidant potential as well as the efficacy(antimicrobials) of the ethanolic extracts of leaves, stem bark and roots of *Boswellia dalzielii* on certain important microbes. **Methodology;** ethanol was used as extraction solvent in this study, total Phenolic content was determine using the famous Folin-Ciocalteu, the antioxidant activity was tested by DPPH (2,2- diphenyl-1-picryl hydrazyl) free radical scavenging method. The agar well diffusion method was used to determine the antibacterial activity of the plant extracts. The MIC of the extracts was determined using broth dilution technique. The results recorded was anaylasis using one-way ANOVA **Results;** Qualitative phytoconstituents profile shows the presence for alkaloids, flavonoids, saponins, tannins, cardiac glycoside, anthraquinone, carbohydrates, steroids and triterpens. Quantitative phytochemical determination of different parts of *Boswellia dalzielii* shows that the total phenolic contents were 433.0 mg of GAE/g, 391.0 mg of GAE/g and 416.0 mg of GAE/g for leave, stem bark and root respectively. The total tannin and Alkaloid contents were 127.7 mg of GAE/g, 117.3 mg of GAE/g, 145.3 mg of GAE/g and 27.2 mg of AE/g, 33.40 mg of AE/g, 42.00 mg of AE/g for leave, stem bark and Root respectively. The result showed that higher zone of inhibition was demonstrated by *Shigella* (23 mm) at concentration of 200 mg/ml for leaves extracts. Lower MIC was recorded in stem bark and was observed in *E.coli*, *Klebsiella pneumonia* and *Shigella* with 12.5mg/ml each while

S.typhi shows the highest with 50mg/ml, 100mg/ml was recorded on leaves extracts for both *E.coli* and *Shigella*, while 50mg/ml was recorded for *Klebsiella pneumoniae* and *S.typhi*. in the root extracts, 12.5mg/ml was recorded for *E.coli*, *Klebsiella pneumoniae* and *Shigella* as lowest while *S.typhi* shows the highest with 25mg/ml

Keywords; *Boswellia dalzielii*, *Ethanol*, *E.coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Shigella specias*

1.0 Introduction

Boswellia dalzielii is a very unique medicinal plant that is part of the Burseraceae family. It has been regarded as a wonder plant in northern part of Nigeria and is called commonly as “ararrabi or hanu” by the hausa speaking communities or frankincense tree in English (Ahmad *et al.*, 2018; Nazifi *et al.*, 2017). All parts of the plant have been reported to have varying medicinal significance. The fresh bark is used to treat giddiness and palpitations (Nwaniyi *et al.*, 2014). It is depended upon by those in the rural areas to ameliorate numerous ailments. It has also been reported to be effective in the management of pains, inflammation, gastrointestinal disorders, fever, ulcer among others (Uzama *et al.*, 2015). To relieve pains, synthetic analgesics have been used, which goes without some forms side effects. At times the cost and availability of such drugs makes it rather difficult for the local populace to manage certain medical conditions. Hence, they still rely on herbs that are readily available, accessible and at no cost other than going in to the bush. Since the use of natural products in the production of some synthetic drugs have been explored severally (Dasilva, 2012) and better understanding of some of the bioactive compounds have taken center stage. The use of more sensitive techniques to detect and evaluate the efficacy of these parts of the plant would be worth re-evaluating. The present study is carried out to evaluate the phytoconstituents, antioxidant potential as well as the efficacy(antimicrobials) of the ethanolic extracts of leaves, stem bark and roots of *Boswellia dalzielii* on certain important microbes. It was observed that most of the reports where backed by qualitative test which this present study sort to deviate from by quantitating the phytoconstituents.

2.0 Materials and Methods

2.1 Reagents

Concentrated Hydrochloric acid, Methanol (CH₃OH) (Guangdong Guanghua Sci. Tech Co., Ltd. Guangdong China, 515000), Ethanol (Guangdong Guanghua Sci-Tech Co. Ltd. Guangdong China, 515000), Sodium hydroxide (NaOH) (Merck Germany), Ferric chloride (FeCl₃), Vanillin reagent, Benedict's reagent, Standard compounds including rutin hydrate, Atropine Sulphate,

Gallic acid, Diosgenin, formic acid, were purchased from Sigma Aldrich of St. Louis, MO. Folin Ciocalteu's Phenol Reagent was purchased from MP Biomedicals of Solon, OH. Wager's reagent, 1,1-diphenyl-2-picrylhydrazyl i.e. DPPH (0.1% w/v in MeOH or EtOH), 1% HCl carotene (0.1% w/v in MeOH or EtOH).

2.2 Sample collection and preparation

The leaves, stem bark and roots of *Boswellia diaziellii* were collected from a farm in Wase local government area of Plateau state (coordinates Latitude: 9° 05'60.00"N, Longitude: 9° 57' 59.99"E). The parts were collected into clean airtight bags, labeled properly and transported to the herbarium section of the department of Botany Ahmadu Bello University Zaria. It was identified and specimen number was obtained (900228) and voucher deposited for referencing.

The samples were air dried under shade, pulverized into fine powder and stored in airtight containers. The various phytochemicals were obtained by drenching 80g of each sample 400 ml of 70% ethanol. The extracting solvent, ethanol has been known as a good solvent for polyphenol extraction and is safe for human consumption. The extract was filtered into freeze drying canisters, dried at -52°C and then stored in a refrigerator at -20°C.

2.2 Total Phenolic Content

The total phenolic content was determined using the famous Folin-Ciocalteu method as describe by Anelise et al, 2014. The data obtain was compared with the standard calibration curve of Gallic acid. Results were presented as milligrams of Gallic acid equivalents (mg of GAE) per gram dry weight (g DW)

2.3 Antioxidant Activity

The antioxidant activity was tested by DPPH (2,2- diphenyl-1-picryl hydrazyl) free radical scavenging method. The free radical scavenging activity of each sample was evaluated with the DPPH free radical scavenging assay (Hyun *et al.*, 2013). Briefly, 0.8 mL of freshly prepared DPPH solution (0.4mM in MeOH) was plated in 96-well plates, and 0.2 ml of the sample (or a control) was added followed by serial dilution to each well. The mixture was incubated for 30 min in the dark at room temperature. Then the absorbance was measured at 520 nm using an iMark™ microplate reader (Bio-Rad). The RC50 (50% reduction of DPPH radicals) was

calculated from a graph of radical scavenging activity versus extract concentration. BHT was used as the standard.

2.4 Antimicrobial Assay

The agar well diffusion method was used to determine the antibacterial activity of the plant extracts. 0.1 ml of the different standardized organism (0.5 Mac Farland) was inoculated on the surface of Mueller Hinton Agar in a sterile Petri dish and allowed to set and then solidified. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 50, 100, 150 and 200 mg/ml while the 5th well contained the solution used for the research to serve as control, Ciprofloxacin (Micro lab limited) 125 mg/ml, was used as control in this research.

These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well, were measured to the nearest millimeters.

2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 200 mg/ml of the extract into a test tube containing 2 ml of Nutrient broth, thus producing solution containing 100 mg/ml of the extract. The process continues serially up to test tube No. 5, hence producing the following concentrations; 100, 50, 25, 12.5, 6.25 mg/ml. Test tube No. 6 do not contain extracts and serve as Control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test organisms were introduced into the test tubes and incubated at 37°C for 24 hours. After incubation the test tubes were observed for growth by checking for turbidity. The least concentration of the extract where there was no growth in tube was taken as the MIC. From each tube that did not show visible growth in the MIC, 0.01ml was aseptically transferred into extract free Mueller Hinton agar plates. The plates were incubated at 37°C for 24hours. The MBC was recorded as the lowest concentration (Highest Dilution) of extract that had less than 99% growth on nutrient agar plates

2.6 Statistical Analysis

The data on the average zone of inhibition produced by the isolates against the extracts used was analyzed using One-Way ANOVAs and the statistical program SPSS 20.0 (Statistical Package for the Social Sciences). The results were presented as the means \pm standard deviation. Significance level for the differences was set at $p < 0.05$.

3.0 Results and Discussion

The result of the qualitative phytoconstituents profile is presented in Table 1. From the table, the different part of the plant tested positive for the presence for alkaloids, flavonoids, saponins, tannins, cardiac glycoside, anthraquinone, carbohydrates, steroids and triterpens.

Table 1: Qualitative profile of phytoconstituents in different parts of *Boswellia dalzielii*

Phytoconstituent	Extract		
	Leave	Stem bark	Root
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Cardiac glycosides	+	+	+
Anthraquinones	+	+	+
Carbohydrates	+	+	+
Steroids and triterpens	+	+	+

Key Positive (+) indicates presence and negative (-) implies absences.

The ethanolic extracts of *Boswellia dalzielii* leave, stem bark and root thus obtained from this study were semi solid in nature, the leave, stem bark and root possess dark green, dark brown and brown color respectively with honey like smell. Percentage yield of the extracts (leave, stem bark and root) were 15.38%, 14% and 4.55% respectively. *Boswellia dalzielii* (*B. dalzielii*) of the frankincense genus is traditionally used in the treatment of rheumatism, pain, and inflammation (Nazifi *et al.*, 2017). Phytochemicals are non-nutritive plants chemical that have protective and diseases preventive properties. These different classes of secondary metabolites have been recognized to possess many pharmacological properties (Nazifi *et al.*, 2017). This

finding is similar to the report of Marius *et al.* (2017) where the methanolic extracts of *Boswellia dalzielii* leaves tested positive for flavonoids, tannins, triterpenoids, cardiac glycosides, and alkaloids while the stem bark tested positive for steroids, glycosides, alkaloids, triterpenoids, carbohydrates, anthraquinones, flavonoids, and saponins. Nazifi *et al.*, 2017; Adalakun *et al.*, 2001) have also reported presence of tannins and Alkaloids in the aqueous extract of stem bark of *Boswellia dalzielii*.

Table 2: Quantitative profile of phytochemicals and antioxidants of *Boswellia diazieli*

Phytoconstituent	Extract		
	Leave	Stem bark	Root
TAC (mg AE/g)	27.20 ± 0.043	33.40 ± 0.067	42.00 ± 0.074
TTC (mg GAE/g)	127.7 ± 0.049	117.3 ± 1.110	145.3 ± 0.087
TPC (mg GAE/g)	433.0 ± 0.043	391.0 ± 0.046	416.0 ± 0.044
TSC (mg DE/g)	0.000 ± 0.00	0.000 ± 0.000	0.000 ± 0.000
DPPH IC₅₀ (µg/ml)	0.982	1.905	2.570
FRAP IC₅₀ (mg/ml)	0.07	0.05	0.03

TAC= Total alkaloid content, TTC= Total tannin content, TPC= Total phenolic content, TSC= Total saponin content, AE= Atropine equivalent, GAE= Gallic acid equivalent, DE= Diosgenin equivalent

The outcomes of the quantitative phytochemical determination of different parts of *B. dalzielii* are presented in Table 2. From the table, the total phenolic contents were 433.0 mg of GAE/g, 391.0 mg of GAE/g and 416.0 mg of GAE/g for leave, stem bark and root respectively. The total tannin and Alkaloid contents were 127.7 mg of GAE/g, 117.3 mg of GAE/g, 145.3 mg of GAE/g and 27.2 mg of AE/g, 33.40 mg of AE/g, 42.00 mg of AE/g for leave, stem bark and Root respectively. The total phenolic contents obtained from this study were thus higher than (373.9 mg of GAE/g.) reported by Yves *et al.* (2018) in their study of methanolic extract of *Boswellia dalzielii* stem bark. Phenolic compounds are secondary plant metabolites found combined with

mono- and polysaccharides, linked to one or more phenolic group, or can occur as derivatives, such as ester or methyl esters. Phenol is attributed to the capacity of scavenging free radicals, donating hydrogen atoms or electrons. This antioxidant mechanism, present in the plants, has an important role in the reduction of lipid oxidation in (plant and animal) tissues, because when incorporated in the human diet, not only it conserves the quality of the food, but it also reduces the risk of developing some diseases. Studies have shown that plant rich in phenolic contents contributes to the delay of the aging process and to the decrease of the inflammation and oxidative stress risk, related with chronic diseases (e.g., cardiovascular diseases, arteriosclerosis, cancer, diabetes, cataract, disorders of the cognitive function, and neurological diseases) Igor (2017). The total tannin contents obtained from this study were similar to those obtained by Yves *et al.* (2018) whose extraction solvents was methanol where the total tannin content obtained was 117.9 mg of GAE/g for *Boswellia dalzielii* stem bark. Tannin compounds are widely distributed in many species of plants, where they play role in protection of plants from predators (including pesticides) and might help in regulating their growth. Many tannin molecules have also been shown to reduce many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules. The anticarcinogenic and antimutagenic potentials of tannins may be related to their antioxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidation. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immunoresponses Chung *et al.* (2005). Alkaloids have a wide range of pharmacological activities including antimalarial (quinine), antiasthma (ephedrine), anticancer (homoharringtonine), cholinomimetic (galantamine), vasodilatory (vincamine), antiarrhythmic (quinidine), analgesic (morphine), antibacterial (chelerythrine), and antihyperglycemic activities (piperine). Alkaloids possess psychotropic

(psilocin) and stimulant activities (cocaine, caffeine, nicotine, theobromine), and have been used in entheogenic rituals or as recreational drugs. Alkaloids can be toxic too (atropine, tubocurarine). Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly evoke a bitter taste (Russo *et al.*, 2013). In the present study, the total alkaloid content obtained for the leave and stem bark of *Boswellia dalzielii* were lower than the result reported by Nwinyi *et al.* (2004) in the aqueous extract of *Boswellia dalzielii* leave (88.5 mg of AE/g) and stem bark (55.6 mg of AE/g). Saponins are diverse group of compounds widely distributed in the plant kingdom, which are characterized by their structure containing a triterpene or steroid aglycone and one or more sugar chains. Consumer demand for natural products coupled with their physicochemical (surfactant) properties and mounting evidence on their biological activity (such as anticancer and anticholesterol activity) has led to the emergence of saponins as commercially significant compounds with expanding applications in food, cosmetics, and pharmaceutical sectors (Li *et al.*, 2010). In this study total saponin contents gave a null value for the leave, stem bark and root. This may be due it's to present in minute quantity that the method could not detect. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Flavonoids have been shown to have a wide range of biological and pharmacological activities. Examples include anti-allergic, anti-inflammatory, antioxidant, anti-microbial (antibacterial, antifungal and antiviral), anti- cancer and anti- diarrheal activities Cazarolli *et al.* (2008). In this study, the total flavonoid contents tested negative for the leave, stem bark and root. This may be due to its present in minute quantity. Instead the result obtained by Yves *et al.* (2018) in the methanolic extract of *Boswellia dalzielii* stem bark was 142.2 mg of RE/g. Natural products of plant origin have been proposed as a potential source of natural antioxidants with strong activity. This activity is mainly due to the presence of phenolic compounds such as flavonoids, phenols, flavonols and pro-

anthocyanidins (Oyedemi *et al.*, 2010). Polyphenols are the major plant compounds with high level of antioxidant activity. This activity could be due to their ability to absorb, neutralize and to quench free radicals (Mathew and Abraham, 2006). Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Oyedemi *et al.*, 2010). According to the food and drug agency (FDA), IC₅₀ represents the concentration of a drug that is required for 50 % inhibition in vitro. The half maximal inhibitory concentration is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance is needed to inhibit a given biological process by half. In other words, it is the half maximal inhibitory concentration of a substance. The IC₅₀ value for the radical scavenging activities of the ethanolic extracts of different part of *Boswellia dalzielii* obtained from this study were 0.982 µg/ml, 1.905 µg/ml and 2.570 µg/ml for leave, stem bark and root respectively. This finding is similar to the result obtained by Alemika *et al.* (2004) in the ethanolic extract of *Boswellia dalzielii* leave (0.985 µg/ml) and stem bark (1.900 µg/ml), higher than the result obtained by Ojerinde (2010) in the methanolic extract of *Boswellia dalzielii* stem bark (1.750 µg/ml) and lower than the result obtained by Yves *et al.* (2018) in the methanolic extract of *Boswellia dalzielii* stem bark (1.990 µg/ml). The DPPH radical scavenging activity of *Boswellia dalzielii* leave, stem bark and root may be attributed to the presence of phenolic and flavonoids contents. The phenolic constituents found in vegetables and spices have received considerable attention due to their antioxidant activity. The ethanolic extracts in this study showed antioxidant activities because the solvent used has a greater affinity in extracting the phytochemical compounds. The iron reducing power of different parts of *Boswellia dalzielii* obtained in this study was determined by measuring the transformation of Fe⁺³ to Fe⁺². The outcome of the present study suggests that the extracts

possessed antioxidant activity in a concentration dependent manner. This effect may suggest the ability of *Boswellia dalzielii* to minimize oxidative damage to some vital tissues in the body Taiwo *et al.* (2004).

Table 3. Antibacterial Activity of *Boswellia dalzielii* Extracts

Extracts	Concentrations mg/ml	Zone of inhibitions (mm)			
		E.coli	Kpn	Staph.. aur	Shig. Sp
STEM BARK	50	11±0.27	12±0.00	09±0.18	11±0.27
	100	12±0.27	12±0.18	10±0.27	13±0.18
	150	15±0.18	16±0.18	13 ±0.27	17±0.27
	200	16±0.27	19±0.27	18±0.27	21±0.00
LEAVES	50	11±0.00	10±0.27	11±0.27	12±0.27
	100	12±0.18	13±0.18	12±0.00	13±0.27
	150	17±0.18	20±0.27	16 ±0.00	19±0.00
	200	20±0.27	21±0.27	19±0.00	23±0.18
ROOTS	50	09±0.27	10±0.00	08±0.27	11±0.00
	100	10±0.18	12±0.00	09±0.27	11±0.27
	150	11±0.27	15±0.27	10±0.18	14±0.27
	200	14±0.00	17±0.27	10±0.18	16±0.27
Control	125	22	23	19	21

KEY

Control = ciprofloxacin 125mg/ml
E.coli= Escherichia coli
Kpn = Klebsiella pneumoniae
Staph. aur = Staphylococcus aureus
Shig. sp = Shigella species

The antibacterial activity of different concentration of *Boswellia dalzielii* extracts against test isolates is presented in Table 3. The result showed that higher zone of inhibition was demonstrated by *Shigella* (23 mm) at concentration of 200 mg/ml for leaves extracts.

Zones of inhibition shown recorded by the control ranges from 19-23 mm.

Table 4; Minimum inhibitory concentration (MIC) of the extracts against the test isolates

EXTRACTS	Isolates/ Minimum inhibitory concentration (mg/ml)			
	<i>E. coli</i>	<i>Klebsiella</i>	<i>S. typhi</i>	<i>Shigella</i>
STEM BARK	12.5	12.5	50	12.5
LEAVES	100	50	100	50
ROOTS	12.5	12.5	25	12.5

The minimum inhibitory concentration of extracts of *Boswellia dalzielii* is represented in Table 4 which shows dilutions of various concentrations of the extracts against test isolates. Lower MIC was recorded in stem bark and was observed in *E.coli*, *Klebsiella pneumoniae* and *Shigella* with 12.5mg/ml each while *S.typhi* shows the highest with 50mg/ml, 100mg/ml was recorded on leaves extracts for both *E.coli* and *Shigella*, while 50mg/ml was recorded for *Klebsiella pneumoniae* and *S.typhi*. in the root extracts, 12.5mg/ml was recorded for *E.coli*, *Klebsiella pneumoniae* and *Shigella* as lowest while *S.typhi* shows the highest with 25mg/ml

Table 5: Minimum bactericidal concentration (MBC) of the extracts against the test isolates

EXTRACTS	Isolates/ Minimum bactericidal concentration (mg/ml)			
	<i>E. coli</i>	<i>Klebsiella</i>	<i>S. typhi</i>	<i>Shigella</i>
STEM BARK	25	50	100	25
LEAVES	50	25	100	50
ROOTS	100	NF	NF	100

Table 5 present the minimum bactericidal concentration (MBC) of the plant extracts. The result shows that the extract of the plant can kill some test isolates at concentration of 25 – 100 mg/ml. However, the MBC of some isolates was unable to be found.

There is no significant different on the activity of different extracts on the tested isolates but significant different exist between the activity of the extracts and that of the standard antibiotic used in the experiment. The result showed that the leaves 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, 1999, 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 ethnobotanical usage of the plant in treating gastroenteritis particularly those caused by the organisms.

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