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Antibacterial activity and phytochemical characterization of *Boswellia dalzielii* Hutch. (Burseraceae) stem bark methanol extract and fractions against selected pathogenic bacteria

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Abstract

The increasing prevalence of antimicrobial resistance has led to a more intensive search for alternative antimicrobial agents from medicinal plants. *Boswellia dalzielii* (Burseraceae) is traditionally used to treat infections, with previous studies reporting antibacterial activity. However, further investigation of its antibacterial properties and bioactive constituents is warranted. This study evaluated the antibacterial activity and phytochemical composition of *B. dalzielii* stem bark extracts against selected pathogenic bacteria. The stem bark was collected, dried, and extracted with methanol using cold maceration. The crude extract was fractionated into *n*-hexane, ethyl acetate, and aqueous fractions. Antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella bongori* was assessed using agar well diffusion method. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by broth dilution. Gas Chromatography-Mass Spectrometry (GC-MS) analysis were performed to identify bioactive compounds. The extracts showed varying antibacterial activity, with the aqueous fraction demonstrating the highest activity (7.50 ± 0.00 mm to 18.45 ± 0.25 mm). MIC values ranged from 18.75 – 150 mg/mL, while MBC indicated bactericidal effects at higher concentrations. The identified compounds, including n-hexadecanoic acid, tetradecanoic acid, and 9,12-octadecadienoic acid, may contribute to the observed antimicrobial activity of *B. dalzielii* and warrant further investigation as potential antimicrobial agents.

Keywords: Antibacterial activity; *Boswellia dalzielii*; GC-MS; Medicinal plants; Phytochemicals

INTRODUCTION

The rapid emergence of antimicrobial resistance has become a major global public health concern, limiting the effectiveness of conventional antibiotics and increasing the burden of infectious diseases. Bacterial pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* species are commonly associated with hospital and community acquired infections, and many of these organisms have developed resistance to multiple antimicrobial agents [1]. This growing resistance has prompted increased research into alternative sources of antimicrobial compounds, particularly medicinal plants which are known to contain diverse bioactive phytochemicals [1,2].

Medicinal plants have been widely used in traditional medicine for centuries, especially in developing countries where access to modern healthcare may be limited. These plants contain secondary metabolites

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such as alkaloids, flavonoids, tannins, saponins, terpenoids, and phenolic compounds that exhibit antimicrobial, anti-inflammatory, antioxidant, and therapeutic properties. Recent studies have highlighted the importance of plant-derived compounds as promising candidates for the development of new antimicrobial drugs due to their structural diversity and reduced side effects [3,4].

B. dalzielii Hutch., belonging to the family Burseraceae, is a medicinal plant widely distributed across Northern Nigeria and other parts of West Africa. The plant is commonly known for its ethnomedicinal uses in the treatment of infections, gastrointestinal disorders, inflammatory diseases, wounds, and fever. Different parts of the plant including stem bark, leaves, and roots have been traditionally used in herbal medicine. Previous phytochemical studies have reported that *Boswellia* species contain biologically active compounds such as terpenoids, flavonoids, phenolics, and essential oils which possess antimicrobial and anti-inflammatory properties [5,6].

Despite the documented traditional use and reported antibacterial activity of *B. dalzielii*, further investigation is warranted to strengthen understanding of its antibacterial effects against clinically relevant pathogenic bacteria. Previous studies have also identified several phytochemical constituents with potential antimicrobial activity; however, further evaluation of their contributions to the observed antibacterial effects is needed. This study therefore aimed to evaluate the antibacterial activity of *B. dalzielii* stem bark extracts against selected pathogenic bacteria using standard microbiological and to determine its phytochemical composition.

EXPERIMENTAL METHODS

Collection and identification of plant materials. Fresh stem bark of *B. dalzielii* was collected from Tunga Wawa at Kontagora (Niger state), authenticated by a taxonomist (Yahaya Adamu) at the herbarium unit of the Department of Biological Science, Federal University of Technology, Minna. The plant material collected was washed under running tap water, air- dried at room temperature in the laboratory and then pulverized and homogenized to fine powder and stored in airtight glass containers for further uses.

Extraction and fractionation of the methanol stem bark of *B. dalzielii.* The powdered stem bark (100g) *of B. dalzielii* was extracted using cold maceration technique with 1 L of methanol in a glass jar for 3 days (72 hours) at room temperature. The extract was filtered; the filtrate was further concentrated by using a rotary evaporator and finally evaporated to dryness using a water bath set at a temperature of 40°C. Crude methanol extract (25 g) was suspended in 100 mL of water and filtered. The filtrate was partitioned thrice with 100 mL of hexane, ethyl acetate and distilled water in increasing order of polarity (n-hexane < ethyl acetate < distilled water) using separating funnel. The crude methanol extract and fractions were evaporated to dryness at a reduced temperature of 40°C over a water bath, labelled appropriately and stored for further use [7,8].

Ethical consideration. Ethical approval was obtained from the ethics and publication committee of General hospital Minna, Niger State with reference number HMB/GHM/136/VOLIII/568 to permit the collection of bacterial isolates.

Isolation and identification of the bacterial Isolates. Clinical bacterial isolates were obtained from the Microbiology Laboratory of General Hospital, Minna, Niger State, Nigeria. Different containers of broth containing the isolates were incubated at 37°C for 24 hours and a loop full of the broth was further sub-cultured onto the agar media which includes the Salmonella-Shigella Agar (SSA), Eosin

Methylene Blue Agar (EMB), and Nutrient Agar (NA) and incubated at 37°C for 24 hours. Colonies from the agar plates were sub-cultured on to nutrient agar and incubated for another 24 hours at 37°C to obtain pure isolates. The pure isolates obtained were identified and characterized by Gram-staining and other biochemical tests [9]. Molecular Characterisation of bacteria isolates was carried out using Polymerase Chain Reaction (PCR). The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer (Applied Biosystems, USA) using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis [10].

Standardisation of the bacterial culture. According to the method described by Iyabo et al. [11], zero point two millilitres (0.2 mL) of overnight cultures of the test organism were transferred into 20 mL of sterile nutrient broth and the culture was incubated for 2-3 hours at 37ºC to standardise the culture to 10^6 CFU/mL McFarland. A loopful of the standardised inoculum was used for the antibacterial assay.

Antibacterial activities. Agar well diffusion method was carried out to determine the antimicrobial activity of extract and fractions as described by Gupta et al. [12]. Mueller Hinton agar plates were prepared for *P. aeruginosa, S. bongori,* and *E. coli.*

Nutrient agar (40 g) was dissolved into 1 L of distilled water in a conical flask. The mixture was sterilized in the autoclave at 121ºC for 15 minutes and the medium was allowed to cool to 45ºC. Sterile molten Muller Hinton agar (20 mL) at 45ºC was dispensed into sterile Petri dishes and allowed to set. A sterile cork borer of diameter 6 mm was used to bore equidistant wells onto the agar plates. One drop of the molten agar was used to seal the bottom of the bored wells to prevent the extract from sipping beneath the agar. Sterile cotton swab sticks were used to streak on the surface of the agar plates with the standardized test organisms and 100 µL of the methanol extract and fractions (15, 25, 50, 100, 150, 200, 250 and 300 mg/mL) stem bark of *B. dalzielii* were added separately to the bored wells and 5 mg/mL of the standard drug (Ciprofloxacin and Amoxicillin) were used as positive control while dimethyl sulfoxide (DMSO) served as the negative control. Thirty minutes pre-diffusion time was allowed after which the plates were incubated at 37°C for 24 hours. The zones of inhibition were then measured in millimetres. The above method was carried out in duplicates, and the mean of the result was taken. Test with ten millimetres. (10 mm) zones of inhibition was considered sensitive to the plant extract.

Determination of Minimum Inhibitory Concentration (MIC). The method of Iyabo *et al.* [11] was employed with slight modification using spectrophotometer to determine the MIC of extract against the organisms. A series of two-fold dilutions of extract and fractions ranging from 300 mg/mL to 38.85 mg/mL was made in nutrient broth. A 0.1 mL portion of each of the standardized test organisms (0.5 McFarland turbidity standards) was added to each dilution. Two controls were maintained for each batch. These included tubes containing extract/fractions and growth medium without inoculum and tube containing the growth medium and inoculum (organism control). The tubes were incubated at 37ºC for 24 hours. At the end of the incubation period, the optical density of the cultures in the test tubes was read using a spectrophotometer at a wavelength of 600 nm. The MIC was determined by subtracting the absorbance of the negative control from the absorbance of the test and comparing the result with the absorbance of the positive. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the extract that produced a marked inhibition of bacterial growth, as indicated by the lowest absorbance value compared with the growth control.

Determination of Minimum Bactericidal Concentration (MBC). The minimum bactericidal concentration (MBC) was determined by sub-culturing the cultures with the lowest optical density beginning with the test tube containing the minimum inhibitory concentration and above onto a freshly prepared nutrient agar medium. The cultures were incubated for 24 hours at 37ºC, after incubation, the culture concentration without visible growth was regarded as the minimum bactericidal concentration.

Gas Chromatography Mass Spectrometer (GC-MS) analysis. The GC-MS analysis of aqueous, ethyl acetate and *n*-Hexane fractions of methanol extract *of B. dalzielii* stem bark, was carried out on a GC-MS-QP 2010 Plus Shimadzu system (SHIMADZU, JAPAN). Gas chromatograph interfaced with a mass spectrometer (GC-MS) instrument was used; Column elite-1 fused silica capillary column (30m x 0.25mm 1D x µL df, composed of 100 % dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1mL/min and an injection volume of 2µL was employed (split ratio of 10:1) injector temperature-250ºC; ion source temperature 280ºC. The oven temperature was programmed from 110ºC (Isothermal for 2 min.) with an increase of 10ºC/min to 200ºC then 5ºC/min to 280ºC/min, ending with a 9 min isothermal at 280ºC. Mass spectra were taken at 70 eV; a scanning interval of 0.5s and fragments from 40 to 550 Da. The total GC running time was 27 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatogram was a turbo mass and the detection of compounds employed the database of the National Institute of Science and Technology (NIST).

Quantitative phytochemical evaluation. The methanol extract of *B. dalzielii* stem bark was analysed quantitatively for the presence of phenols, flavonoids, tannins, and saponins using spectrophotometric method.

Determination of total phenolic content (TPC). Total phenol content in methanol extract *of B. dalzielii* stem bark was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using gallic acid as the standard and expressing results as Gallic Acid Equivalent (GAE) per gram of sample [13]. Different concentrations (0.01 - 0.1 mg/mL) of Gallic acid were prepared in methanol. Aliquots of 0.5 mL of the test sample and each sample of the standard solution were taken, mixed with 2 mL of Folin–Ciocalteu reagent (1:10 in deionised water) and 4 mL of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubate at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. All the samples were analysed in three replications. The total phenol was determined with the help of standard Curve prepared from pure phenolic standard (Gallic acid) [13].

Determination of total flavonoid content (TFC). The TFC of methanol extract *of B. dalzielii* stem bark was determined by aluminium chloride colorimetric assay [14]. Briefly, 0.5 mL aliquots of the methanol extract and standard solution (0.01 – 0.1 mg/mL) of Quercetin were mixed with 2 mL of distilled water; and subsequently with 0.15 mL of sodium nitrite (5% $NaNO_2$, w/v in methanol). After 6 minutes, 0.15 mL of (10% $AlCl_3$, w/v in methanol) solution was added. The solution was allowed to stand for 6 min and after that 2 mL of sodium hydroxide (4% NaOH, w/v in methanol) solution was added to the mixture. The final volume was adjusted to 5 mL with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 minutes. The absorbance of each mixture was determined at 510 nm. TFC was determined as mg Quercetin equivalent per gram of sample with the help of calibration curve of Quercetin.

All determinations were performed in triplicate.

Determination of tannin content. The tannins were determined by Folin-Ciocalteu method described by [16]. The sample extract (0.1 mL) was added to a 10 mL volumetric flask containing 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu-phenol reagent, 1 mL of 35% Na_2CO_3 solution and diluted to 10 mL with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of Gallic acid (0.01 – 0.1 mg/mL) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract [15].

Determination of saponin content. Total saponins were determined according to the method described by [16]. Freeze-dried extract (1 g) was dissolved in 50% aqueous methanol, and an aliquot of 0.25 mL (5 mg/mL) was taken. Vanillin reagent (0.25 mL; 8%) was added followed by sulphuric acid (2.5 ml; 72% v/v). The reaction mixtures were mixed well and incubated at 60°C on a water bath for 10 min. After incubation, the reaction mixtures were cooled on ice and absorbance at 544 nm (UV visible spectrophotometer) was read against a blank that does not contain extract. The standard calibration curve was obtained from aliquots of diosgenin (0.01 – 0.1 mg/mL in 50% aqueous methanol). The total saponin concentration was expressed as mg diosgenin equivalents (DE) per g dry weight (DW).

Data analysis. Data generated were expressed as mean value ± standard error of mean (SEM). Among groups, comparisons of means were performed by two-way ANOVA test for statistical significance of differences at $p<0.05$. Mean values were separated by Duncan multiple Range Test (DMRT). All data were evaluated using the statistical package SPSS version 25.0.

RESULTS

Characterisation and identification of bacterial isolates. Morphological and biochemical tests results are displayed in Table 1 and 2.

Polymerase chain reaction amplifications of total genomic DNA of the bacteria isolates using primer pair 27F 5'-AGA GTT TGA TCM TGG CTC AG-3' and -1525R, 5′-AAGGAGGTGATCCAGCC-3′ primers produced a PCR product of about 1500 base pairs (bp) (Plate I). The PCR products were sequenced (Gen Bank Accession Number: MK572634.1, MN623691.1 and CP074120.1for *P. aeruginosa*, *S. bongori*, and *E. coli* respectively) (Table 3). The phylogenetic tree of the isolates shows the relationship among the organisms and their origin using the NCBI data (Figure 1).

Antibacterial evaluation of crude extracts and their fractions. The crude methanol extract of the plants extract and fractions of *B. dalzielii* stem were found to exhibit appreciable antibacterial effects on the bacteria isolates (*E. coli, P. aeruginosa* and *S. bongori)* at concentration of 150 to 300 mg/mL. The crude methanol extract of *B. dalzielii* stem shows more activity against *E. coli* and *S. bongori* with inhibition zones ranging from 5.50 ± 0.0 to 18.55 ± 1.12 and 1.50 ± 1.5 to 15.05 ± 0.05 mm respectively, however, the least activity was observed for *P. aeruginosa* (6.70 ± 0.03 to 12.80 ± 0.20 mm). The standard drugs (Amoxillicin and ciprofloxacin) used inhibited the growth of all the isolates with varying zones of inhibition ranging from 7.30±1.1 to 24.35±1.50 mm (Table 6). The n-hexane fraction shows a narrow zone of inhibition against all the bacterial isolates. The residual aqueous fractions show significant inhibition against *P. aeruginosa, E. coli* and *S. bongori* at both 250 mg/mL and 300 mg/mL

with inhibition zones ranging from 5.00±1.00 to 19.50±0.50 mm. The ethyl acetate fraction of the stem extract has significant activity against *E. coli* at (9.00±1.00 to17.50±0.50 mm) (Table 7).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of Methanol extract and fractions of *B. dalzielii* stem bark. The result of the antimicrobial activities of the extracts as determined by measuring the minimum inhibitory concentrations (MIC) of methanol extract and fractions of *B. dalzielii* stem bark are presented in Figures 3 to 6 respectively. In present study, MIC values ranged between 9.38 - 150 mg/mL and most of the cases were in correlation with the antibacterial activity determined by inhibition zone formation.

GC-MS analysis of fractions from *B. dalzielii* stem bark. The compounds present in the fraction of *B. dalzielii* stem bark were identified by GC-MS analysis. The active compounds with their retention time (RT), molecular formula, molecular weight (MW) and peak area (%) are presented in Table 8. The major constituents identified in the three fractions are 9-Octadecenal (53.78%), Hexadecanoic acid (22.82%), Octadecanioc acid or Stearic acid (10.68%), 2-methylnonane (3.42%) and 9, 12-Octadecadenoic acid or Linoleic acid (2.84%).

Quantitative phytochemical analysis. Quantitative analysis of the phytochemicals content revealed phenol to be in high concentration in methanol extract of *B. dalzielii* stem bark. The concentrations of the total phenols, saponins, tannins and flavonoids was observed to be 989.04 ± 0.60, 544.22 ± 0.46, 141.58 ± 0.50, and 25.32 ± 0.25 respectively (Table 9).

DISCUSSION

The isolates were characterised using morphological characteristics (Table 1 and 2). Isolates in this study displayed the typical morphological characteristics for *E. coli, P. aeruginosa and S. bongori.* The test isolates used in this study were identified and confirmed prior to the evaluation of the antibacterial activity of *B. dalzielii* stem bark extract and its fractions. The antibacterial activity observed against *E. coli, P. aeruginosa* and *S. bongori* is therefore discussed in relation to the concentration of the extracts and fractions, the susceptibility of the individual test organisms, and the phytochemical constituents identified in the plant material.

Table 1: Cultural growth characteristics on media, microscopic morphological characteristics and Gram staining

Organism	GC & M	MCM	Gram staining
E. coli	Greenish metallic sheen colonies on EMB	Rod shaped bacilli	Negative
P. aeruginosa	Large, flat, opaque colonies with a characteristic blue-green pigment	Rod shaped bacilli	Negative
S. bongori	Colourless colonies with black centre on SSA	Rod shaped bacilli	Negative

GC & M = growth characteristics on media, MCM= microscopic morphological characteristics

Table 2: Biochemical characteristics of bacteria isolates

Organism	Indole	Methyl red	Voges–Proskauer	Citrate	Catalase	Oxidase	Urease
E. coli	+	+	-	-	+	-	-
P. aeruginosa	-	-	-	+	+	+	-
S. bongori	-	+	+	-	+	-	-

+ = Positive - = Negative

Table 3: Molecular characterisation of bacteria isolates

Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
P. aeruginosa	287	2623	99%	0	99.93%	1473	MK572634.1
S. bongori	54736	2647	99%	0	99.52%	1526	MN623691.1
E. coli	562	2630	99%	0	99.58%	4844032	CP074120.1

IDZU7= *P. aeruginosa*, IDZU8= *S. bongori*; IDZU9 = *E. coli*

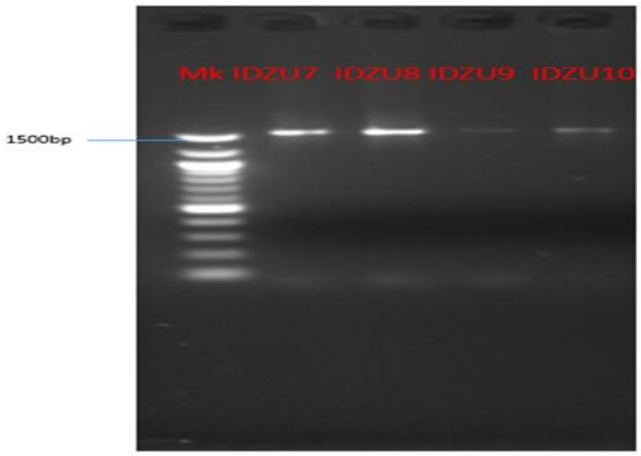
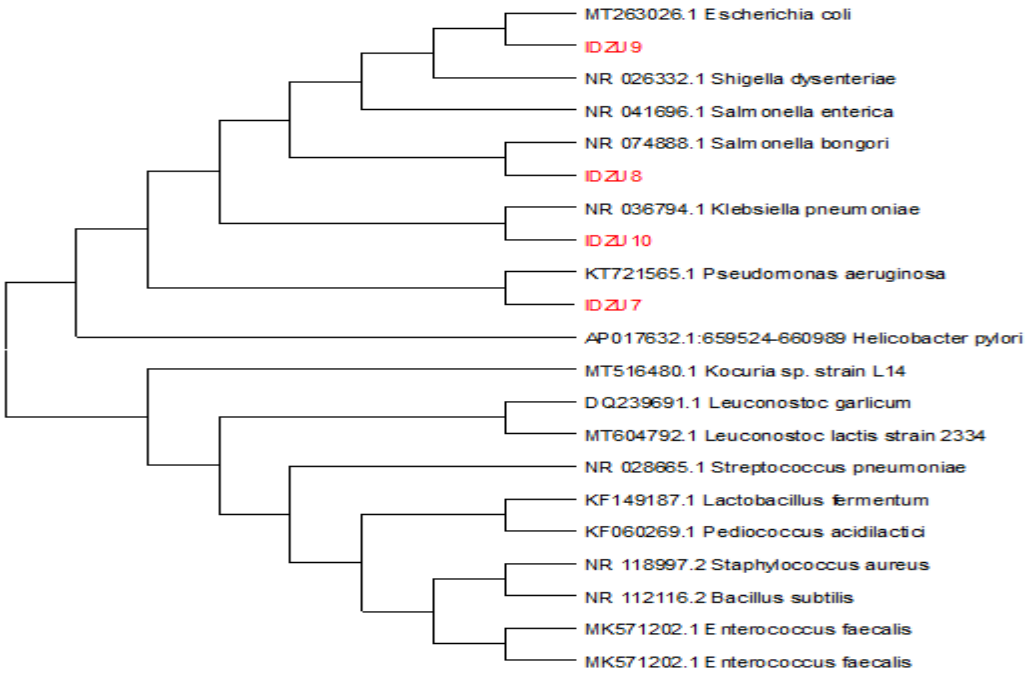


Figure 1: PCR amplification of the 16S *r*RNA gene of bacterial isolates using the universal primers 27F and 1525R. Lane MK represents the 1500 bp DNA ladder, while lanes IDZU7, IDZU8, IDZU9 and IDZU10 represent DNA samples from the respective bacterial isolates subjected to PCR amplification



IDZU7 = *P. aeruginosa,* IDZU8 = *S. bongori* and IDZU9 = *E. coli*

Figure 2: Phylogenetic tree showing the evolutionary relationships among the bacterial isolates based on 16S *r*RNA gene sequences. Isolate codes IDZU7, IDZU8 and IDZU9 were identified as *P. aeruginosa*, *S. bongori* and *E. coli*, respectively

Table 4: Mean zones of inhibition of methanol extract of *B. dalzielii* stem bark against bacterial isolates

Organisms	100	150	200	250	300	Cipro 5 mg/mL	Amox 5 mg/mL
E. coli	7.50±0.00^{b}	10.00±0.00^{c}	13.05±0.05^{d}	14.50±0.50^{d}	18.45±0.25^{e}	18.55±1.12^{e}	3.10±0.2^{a}
P. aeruginosa	-	-	6.70±0.30^{a}	10.30±0.30^{b}	12.80±0.20^{c}	24.35±1.50^{d}	12.75±0.22^{c}
S. bongori	-	-	1.50±1.50^{a}	13.85±0.15^{c}	15.05±0.05^{d}	23.01±0.20^{e}	7.30±1.11^{b}

Values are expressed as mean zone of inhibition (mm) ± standard deviation of replicate determinations. The numbers 100, 150, 200, 250 and 300 represent the concentrations of the plant extract tested (mg/mL). Ciprofloxacin (Cipro) and amoxicillin (Amox) were used as reference antibiotics at a concentration of 5 mg/mL. Different superscript letters (a–e) within the same row indicate statistically significant differences between mean values at $p < 0.05$, whereas mean values sharing the same superscript letter are not significantly different. The symbol (–) indicates no detectable zone of inhibition

Table 5: Mean zones of inhibition of *B. dalzielii* stem bark extract and fractions against bacterial isolates

Organism	H250	H300	A150	A250	A300	E150	E250	E300
E. coli	6.50±0.50^{a}	10.50±0.50^{b}	11.50±1.50^{b}	17.50±0.50^{c}	19.50±0.50^{e}	9.00±1.00^{b}	15.50±0.50^{d}	17.50±0.50^{c}
P. aerugino	-	-	-	8.50±0.50^{b}	11.50±0.50^{c}	5.55±1.00^{a}	6.50±0.50^{a}	8.50±0.50^{b}
S. bongori	-	-	-	5.00±1.00^{a}	7.00±1.00^{a}	6.90±0.0^{a}	8.50±0.50^{b}	11.50±0.50^{c}

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ($p > 0.05$), n=3, - = No activity. H: n-Hexane fraction, A: Residual aqueous fraction, E: Ethyl acetate fraction. Different superscript letters (a–e) within the same row indicate statistically significant differences between mean values at $p < 0.05$, whereas mean values sharing the same superscript letter are not significantly different

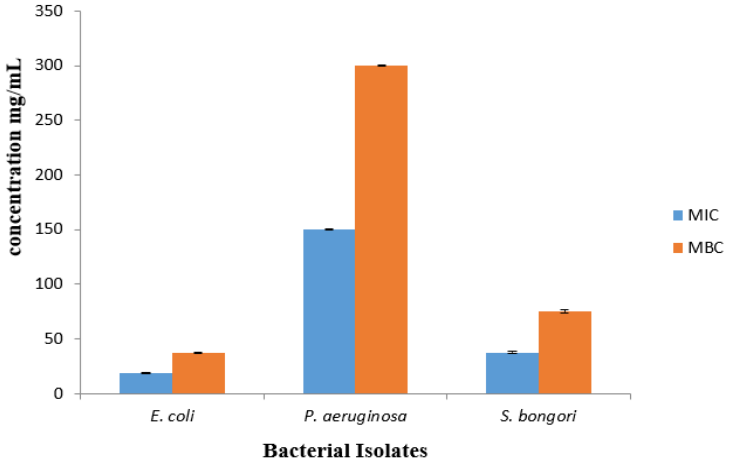


Figure 3: MIC and MBC of methanol crude extract of *B. dalzielii* stem bark
Bar of same color with the same alphabet have no significant difference at $p < 0.05$

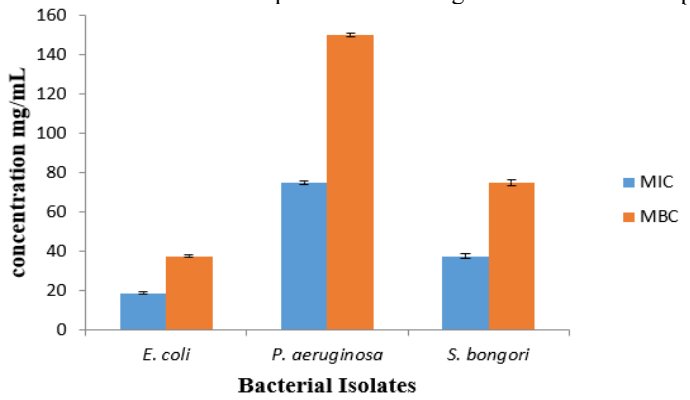


Figure 4: MIC and MBC of aqueous fraction of *B. dalzielii* methanol stem bark extract
Bar of same colour with the same alphabet have no significant difference at $p < 0.05$

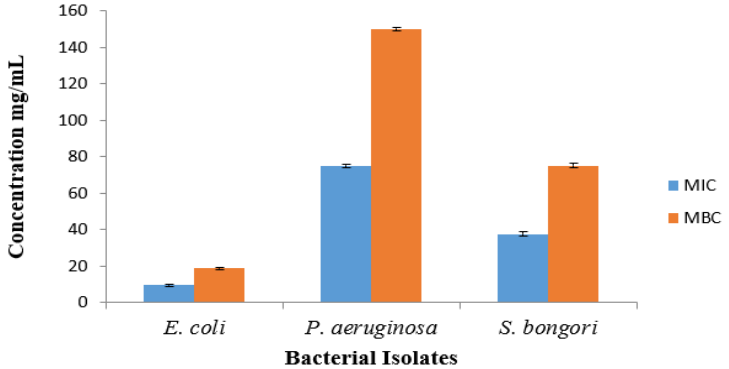


Figure 5: MIC and MBC of n-Hexane fraction of *B. dalzielii* methanol stem bark extract
Bar of same colour with the same alphabet have no significant difference at $p < 0.05$

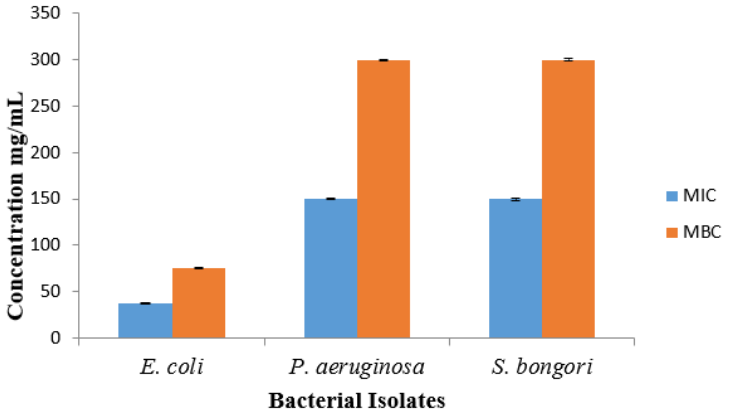


Figure 6: MIC and MBC of ethyl acetate fraction of *B. dalzielii* methanol stem bark extract
Bar of same colour with the same alphabet have no significant difference at $p < 0.05$

Table 8: GC-MS profiles of the fractions of *B. dalzielii* stem bark

	PN	RT	Compound names	MF	MW	% Conc.
Aq	9	14.899	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	4.02
	13	16.156	Tetradecanoic acid (Myristic acid)	$C_{14}H_{28}O_2$	228	23.94
	14	16.188	9,12 Octadecadienoic acid (Z,Z)-	$C_{19}H_{34}O_2$	294	20.38
	17	18.746	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl	$C_{19}H_{38}O_4$	330	6.42
	20	20.057	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl	$C_{21}H_{38}O_4$	354	50.05
Et	26	14.756	1,2-Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	334	5.90
	27	15.019	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	12.02
	29	16.217	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	20.50
	30	16.425	9-Octadecenamide, (Z)-	$C_{18}H_{35}NO$	281	6.31
	34	19.035	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	5.27
	37	20.030	E,Z-1,3,12-Nonadecatriene	$C_{19}H_{34}$	262	12.85
Hex	15	14.940	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.78
	16	15.084	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	5.11
	21	16.139	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{36}O_2$	280	5.46
	23	16.253	Cyclopropaneoctanoic acid	$C_{22}H_{38}O_2$	334	4.51
	25	16.379	2,2-Dimethyl-1-(3-oxo-but-1-enyl) cyclopentanecarboxaldehyde	$C_{12}H_{18}O_2$	194	23.54

Aq = Aqueous residual fraction; Et = Ethyl acetate fraction; Hex = *n*-Hexane fraction

Table 9: Quantitative phytochemical contents of methanol extracts of *B. dalzielii* stem bark

Metabolites	MEBDS (mg/g)
Total Phenols	989.04 ± 0.60
Total Flavonoids	25.32 ± 0.25
Total Tannins	141.58 ± 0.50
Total Saponins	544.22 ± 0.46

MEBDS: Methanol extract of *B. dalzielii* stem bark. Values represent the quantitative phytochemical contents of the methanol extract only; no fractions were included in this analysis. Results are expressed as mean ± standard deviation (SD)

The methanol extract and fractions demonstrated antibacterial activity against *E. coli, P. aeruginosa* and *S. bongori*, although the magnitude of activity varied among the extracts and test organisms. The methanol extract exhibited the broadest antibacterial activity, producing zones of inhibition of 18.45 ± 0.25 mm against *E. coli*, 12.80 ± 0.20 mm against *P. aeruginosa*, and 15.05 ± 0.05 mm against *S. bongori* at 300 mg/mL (Table 4). Among the fractions, the aqueous fraction showed greater activity than the ethyl acetate and n-hexane fractions, with zones of inhibition of 19.50 ± 0.50 mm against *E. coli*, 11.50 ± 0.50 mm against *P. aeruginosa*, and 7.00 ± 1.00 mm against *S. bongori* at 300 mg/mL (Table 5). In contrast, the n-hexane fraction showed no detectable activity against *P. aeruginosa* and *S. bongori*

at the tested concentrations and only limited activity against *E. coli* (Table 5).

The ethyl acetate and n-hexane fractions exhibited comparatively lower antibacterial activity than the methanol extract and aqueous fraction (Table 5). At 300 mg/mL, the ethyl acetate fraction produced zones of inhibition of 17.50 ± 0.50 mm against *E. coli*, 8.50 ± 0.50 mm against *P. aeruginosa* and 11.50 ± 0.50 mm against *S. bongori*, whereas the n-hexane fraction produced a zone of 10.50 ± 0.50 mm against *E. coli* and showed no detectable inhibition against *P. aeruginosa* and *S. bongori*. In comparison, the aqueous fraction produced zones of 19.50 ± 0.50 mm, 11.50 ± 0.50 mm and 7.00 ± 1.00 mm against *E. coli*, *P. aeruginosa* and *S. bongori*, respectively, at 300 mg/mL (Table 5).

The diameter of the zone of inhibition was used to assess the antibacterial activity of the extracts and fractions against the test organisms. *P. aeruginosa* and *S. bongori* showed no detectable inhibition by the n-hexane fraction at the tested concentrations, whereas both organisms showed susceptibility to the aqueous and ethyl acetate fractions and the methanol crude extract (Tables 4 and 5). The methanol extract demonstrated broad-spectrum antibacterial activity, as all three test organisms were inhibited at 200, 250 and 300 mg/mL, with the zones of inhibition increasing with concentration (Table 4).

The methanol crude extract and residual aqueous fraction of *B. dalzielii* stem bark demonstrated greater antibacterial activity against the test organisms than the ethyl acetate and n-hexane fractions, as indicated by their larger zones of inhibition at the tested concentrations (Tables 4 and 5). The observed activity suggests that the methanol extract and residual aqueous fraction contain constituents with antibacterial properties that may contribute to the inhibition of the test organisms under the conditions of this study.

It was observed that the Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts varied in most cases (Figure 2 to 5). The MIC values obtained for the entire test organisms ranged from 18.75 mg/mL to 150 mg/ml. The MBC in most cases varied from the MIC, indicating a different concentration needed to inhibit the growth of the microorganisms and an entirely different one to kill them. From the results of this study, *B. dalzielii* stem bark possesses promising antimicrobial activities against the test organisms.

The MIC is usually described as the lowest concentration of an antimicrobial agent that results in inhibition of visible growth (that is colonies on an agar plate or turbidity in broth culture) under standard conditions [19]. The basic quantitative measures of the In-vitro activity of antibiotics and plant extracts with antibacterial potentials are the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [19]. The MIC values obtained for the entire test organisms, ranged from 18.75 mg/mL to 150mg/mL, when compared to the values of 0.01 - 10 µg/mL usually recorded for typical antibiotics. This difference may be because the extracts and the fractions as well were in impure forms and would contain substances which do not have antibacterial activities [20]. It was observed that the Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts of *B. dalzielii* stem varied in most cases (Figure 2 to 5). The MBC (Figure 2 to 5) in most cases varied from the MIC, indicating a different concentration needed to inhibit the growth of the microorganisms and an entirely different one to be bactericidal.

The GC-MS analysis provided a chemical profile of the compounds detected in the methanol extract and solvent fractions of *B. dalzielii* stem bark. Several of the identified compounds have previously been reported to possess biological activities, including antimicrobial, antioxidant and anti-inflammatory properties. The occurrence of

such compounds may provide a possible chemical basis for some of the biological activities observed in the present study. However, the identification of these compounds by GC-MS alone does not establish that they are responsible for the antibacterial activity observed, and therefore the GC-MS findings should be interpreted as a chemical characterization of the extracts rather than direct validation of the ethnobotanical uses of *B. dalzielii* (Table 8).

The major constituents with high percentage concentration include 9,12-Octadecadienoic acids (Z, Z)- 2,3-dihydroxypropyl (50.05%), 9-Hexadecenoic acid (23.38%), Tetradecanoic acid (23.94%). others with low concentration identified were n-Hexadecanoic acid (4.02%), 9,12 Octadecadienoic acid (Z, Z) (4.83%), hexadecadienoic acid, 2-hydroxy-1-(hydroxymethyl) (6.42%). In addition, 6 compounds were identified from the ethyl acetate fraction of *B. dalzielii* stem which include 9,12 Octadecadienoic acid (Z, Z) (20.50%), E, Z-1,3,12-Nonadecatriene (12.85%), n-Hexadecanoic acid (12.02%), 9-Octadecenamide, (Z)- (6.31%), 1,2-Benzenedicarboxylic acid, butyl octyl ester (5.90%) and Bis (2-ethylhexyl) phthalate (5.27%). Furthermore, 5 compounds were identified from the n-hexane fraction of *B. dalzielii* stem which include n-Hexadecanoic acid (5.78%), Hexadecanoic acid, ethyl ester (5.11%), 9,12 Octadecadienoic acid (Z, Z) (5.46%), and Cyclopropane octanoic acid (4.51%).

About 70% of the major components of residual aqueous fraction, ethyl acetate fraction and n-Hexane fraction of *B. dalzielii* stem bark include n-Hexadecanoic acid which is commonly known as Palmitic acid has been reported to have lubricant, flavour, haemolytic 5-alpha reductase inhibitor, nematicide, anti-androgenic, pesticide, antioxidant and hypo-cholesterolemic properties [21]. n-Hexadecenoic acid which is a fatty acid ester also have nematicide, pesticide, lubricant, anti-androgenic, flavor, and has hemolytic 5-alpha reductase inhibitor properties [22] and (z)-9-Octadecenoic commonly known as Oleic acid which is the major component of unsaturated fatty acid that is responsible for its antimicrobial activity, strong antioxidant and anticancer activity [23]. Furthermore, the extract has been shown to contain 1,2-Benzenedicarboxylic acid, butyl octyl ester which possess antimicrobial and antifouling properties, Bis (2-ethylhexyl) phthalate has antimicrobial activity [24]. Also, E, Z, 1,3,12 nonadecatriene possess anti-inflammatory and antioxidant properties [25]. However, the differences in the concentrations of the bioactive compounds identified across the aqueous, ethyl acetate, and n-hexane fractions may be attributed to the differences in solvent polarity, which influence the selective extraction and partitioning of phytochemical constituents from the stem bark of *B. dalzielii* [26,27].

The total flavonoid content of the methanol extract of *B. dalzielii* stem bark was 25.32 mg/100 g. Flavonoids are considered important natural phenolic compounds because of their broad range of chemical and biological activities, including antioxidant and free radical-scavenging properties [28]. These properties may contribute to their reported antimicrobial, anti-allergic, and anticancer activities. In addition, other phytoconstituents, such as tannins and polyphenols, possess antioxidant and free radical-scavenging activities, which may help protect cells against oxidative damage and may contribute to the prevention or management of certain diseases associated with oxidative stress [29] and these phenolic compounds, which include phenol, tannin and flavonoids, have been found in appreciable amounts in *B. dalzielii* stem.

The presence of tannin is also important, as it forms irreversible complexes with prolin-rich protein, which results in protein synthesis inhibition [30] as well as .

reactions with proteins to provide tanning effect that helps in the treatment of inflamed ulcerated tissues. Most herbs that contain tannin as a major constituent are claimed to be astringent in nature and useful in the treatment of intestinal disorders like diarrhea and dysentery, wounds, sprains, bruises and arresting bleeding [31].

Saponins from plants have long been employed for their detergent properties. It is used as mild detergents and in intracellular histochemistry staining to allow antibody access to intracellular proteins [32].

Conclusion. This study demonstrated that *B. dalzielii* stem bark possesses notable antibacterial activity against *E. coli*, *P. aeruginosa*, and *S. bongori*. The methanol crude extract and aqueous fraction exhibited the highest antibacterial effects, while the n-hexane fraction showed the least activity. The observed antimicrobial activity may be as a result of the presence of bioactive phytochemicals such as phenols, flavonoids, tannins, and saponins, as well as several biologically active compounds identified through GC-MS analysis. These findings highlight its potential as a source of novel antimicrobial agents.

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