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ORIGINAL ARTICLE

Toxicological studies and bioactivity-guided identification of antimicrobially active compounds from crude aqueous stem bark extract of *Boswellia dalzielii*

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ABSTRACT

Objective: The main objective of this study is to isolate, identify, and quantify the active antimicrobial compounds present in the crude aqueous stem bark extract of *B. dalzielii* using some common pathogenic microorganisms as well as toxicological profile.

Material and Methods: Crude aqueous stem bark extract of *Boswellia dalzielii* (CASEB) was partitioned by preparative thin layer chromatography (PTLC) using chloroform–methanol–water, 8:2:1 (v/v). The resulting bands were extracted using chloroform–methanol (50:50). The extract of each band was evaluated for antimicrobial activity on *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, and *Candida albicans* by disc diffusion. Compounds in the most antimicrobially bioactive fraction (MAAF) were identified by high performance liquid chromatography (HPLC), Fourier transform infrared spectrophotometry (FT-IR), and gas chromatography-mass spectrometry (GC-MS). Toxicological profile of the CASEB was evaluated by studying its effect in albino Wister rats.

Results: PTLC produced five bands/fractions of which the MAAF was identified as RF_2-fraction being active against all the isolates except *E. coli* and *K. pneumoniae.* HPLC of the MAAF revealed seven components; FT-IR revealed 17 functional groups; GC-MS revealed five compounds of which 93.18% are Oleic acid (44.88%), Squalene (34.16%), and n-Hexadecanoic acid (14.14%). The acute toxicity showed LD_{50} > 3,000 mg/kg. Sub-chronic toxicity showed that higher doses of the CASEB caused significant changes in liver function indices and a fatty change with lymphocytic infiltration (sign of acute hepatitis) in the liver tissues, but none of these changes were observed in the kidneys.

Conclusion: The antimicrobially active compounds in CASEB were Oleic acid, Squalene, and n-Hexadecanoic acid. These can be further purified and used as precursors of new antimicrobial agents for treating infections especially those due to fungi and *Pseudomonas* spp. that are known to resist wide array of antimicrobial agents. The LD_{50} of CASEB is >3,000 mg/kg in rats. However, long-term consumption of CASEB is associated with significant liver damage.

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Introduction

Herbal plants possessed useful medicinal properties, which were first medicines, is a worldwide phenomenon. For many decades, plants have been used by man to treat various ailments [1–4]. Today, most people still depend on medicinal plants for their health care needs [5]. This may not be unconnected with the fact that plants, as opposed to most synthetic products, have better cultural

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acceptance and satisfactoriness, better synergy or compatibility (tolerability) with the human body [6], fewer side effects or lesser toxicity [7], better protection [7] and most importantly higher spectrum of activity [8] due to their multi-component parts that act synergistically to make them not easily resisted by pathogenic microorganisms. Conversely, many chemically synthetic antimicrobial agents are associated with high cost [9,10] and many side effects in the body such as hypersensitivity, allergic reactions, and immune-suppression [4,11]. Hence, the need to search for more medicinal plants as alternative treatment can never be over emphasized.

Boswellia dalzielii plant currently represents an interesting area of pharmaceutical research due to its several important pharmacological properties. It is popularly used in ethnopharmacopoea for the therapy of numerous human diseases such as venereal diseases, fever, rheumatism, snake bite, septic sores, diarrhea, and other gastrointestinal ailments [12]. Several studies have reported that *B. dalzielii* possesses many pharmaceutical properties such as anti-inflammatory activity [13–15], antioxidant activity [16–18], anticancer activity [12,15,19], anti-ulcer activity [20], hepatoprotective activity [12,19], anti-Alzheimer [15], anti-neuropathic pain (antinociceptive) activity [21], antihyperuricemia [15], and broad spectrum of antimicrobial activity [12,19,20].

However, despite all the above important pharmacological functions, Abdulazeez et al. [22] observed a dose-dependent increase in liver function indexes in rat models pre-treated with aqueous extract from stem bark of *B. dalzielii.* In addition, Salisu et al. [19] reported that the ethanolic extract contains some potentially toxic chemical compounds that may render the plant unsafe for consumption.

Therefore, further research is necessary to determine the full identities and quantities of the main bioactive compounds present in the stem bark of this plant, as well as in-depth toxicity studies to determine the full toxicological characterization of the stem bark of the plant to ascertain its safety for consumption purpose. This study therefore was aimed at isolation, identification, and quantification of the antimicrobially active compounds present in the aqueous stem bark extract of *B. dalzielii* using advanced techniques such as plate thin layer chromatography (PTLC), gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectrophotometry (FTIR), high performance liquid chromatography (HPLC), and contact bioautography using some common human pathogenic microorganisms, which included *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi*, and *Candida albicans*. In addition, the study also determined the full toxicological profile of the plant by evaluating its effect upon acute and sub-chronic administration in albino Wistar rats with the aid of advanced automated techniques, hence, the significance of this research and its contribution to knowledge.

Materials and Methods

Plant material

The stem bark of *B. dalzielii* plant was obtained from Kafur Local Government area of Katsina State, Nigeria. It was taken to the Department of Plant Biology, Bayero University Kano, Nigeria, where it was identified and confirmed at the herbarium section.

Experimental animals

Fifty female adult albino Wistar rats weighing between 120 and 150 gm were used. They were kept under standard humidity (40%–70%), temperature (23°C–25°C), and light (12-h light/darkness cycle) in the animal house, provided standard feed for experimental rodents and had free access to water. The animals were let to acclimatise for seven days prior to the start of the study. The study was carried out following the guidelines of the Good Laboratory Practice Regulations, as recommended by WHO [23].

Ethical issues

This research was fully funded by the Umaru Musa Yar'adua University-based TetFund Research Grant (UMYU/UBR/2017). All the methods used in this research and ethical issues were fully reviewed and approved by the Faculty-based Research Committee and University's Central Research Committee.

Test isolates

The clinical isolates of the bacteria and fungi used for this study were obtained from Aminu Kano Teaching Hospital, Kano. They included *S. aureus*, *S. pyogenes*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, *P. mirabilis*, *S. typhi*, and *C. albicans*. They were characterized using the methods of Cheesbrough [24] by observing their cultural growth characteristics and testing their biochemical characteristics.

Extraction of the crude extract

The stem bark sample was extracted based on the recommendation of Salisu et al. [12,19]. The stem bark was thoroughly washed with tap water, air-dried at room temperature in the laboratory, pulverized to fine powder, and subsequently extracted in sterile distilled water (50-gm stem bark powder: 500 ml of water) by percolation method with vigorous shaking at periodic intervals. The mixture obtained was filtered using muslin cloth and then re-filtered by passing through Number 1 Whattman's filter

paper. The solvent in the filtrate was evaporated on water bath at 45°C to get the crude extract which was transferred into clean sterile airtight glass container and preserved in the refrigerator at 4°C before downstream application.

Bioassay studies

Plate thin layer chromatography

Plate TLC (P. TLC) was carried out on the crude extract to determine the best solvent system that can be used to fractionate the extract in the main preparative TLC. A 0.5 mg/ml solution of the crude extract was prepared using sterile distilled water and subsequently applied on TLC micro slides of aluminum sheath (2 × 20 cm) pre-coated with silica gel of 1.0-mm thickness (one dot per slide). The dotted extract on the various slides were partitioned using different solvent systems of varying polarities ranging from highly polar to non-polar solvents system combinations until the solvent system of chloroform, methanol, and water (CMW) at a ratio of 95:9:1, which provided maximum separation of components along the aluminum coated silica gel slide, visualized under UV light, was obtained.

Preparative thin layer chromatography

PTLC was carried out using commercially developed glass coated silica gel TLC plates of 20 cm × 20 cm sizes to fractionate the extract into its various components using the CMW solvent system discovered from the P. TLC analysis. A pencil was used to draw a horizontal line 2 cm from the base of each plate. Then 0.5 mg/ml of the extract was prepared in sterile glass vial and dotted closely along the horizontal base line of each plate, making approximately 40 dots of the extract per plate. The plates were allowed to dry for 30 min for the solvents to evaporate before putting them in the TLC tanks containing 200 ml of the CMW solvent system and allowed sufficient time for the solvents to move and carry the extract upward along the TLC plates for the separation to take place, after which the plates were dried at room temperature for 24 h and then viewed under UV light. The bands for each plate were marked and their RF values were calculated and recorded. Each band was scraped into a sterile beaker, weighed, and extracted using chloroform–methanol solvent (50:50) to obtain the pure fractions of the extract which were weighed and stored in the refrigerator at 4°C until needed for downstream application.

Determination of the most bioactive component(s) in the extract by contact bioautography of the TLC fractions

Direct contact Bioautography assay was carried out using the pure TLC fractions of extract to test their antimicrobial activity. Two milliliters (2 ml) of dimethyl sulfoxide were added to each of the dried TLC fractions to dissolve them. Labeled Sterile Wattman's filter paper discs were soaked in each solution for 5 min to absorb the components, removed and dried, and then used in evaluating the antimicrobial activity of each fraction on the test organisms using the Kirby-Bauer agar disc diffusion method described by Cheesbrough [24]. The activity was evaluated by the presence or absence of zone of inhibition around each disc. The most bioactive fraction was identified as the fraction with the highest antimicrobial activity.

Identification of compounds in the most bioactive TLC fraction of the extract

The most Bioactive TLC fraction of the extract was subjected to HPLC, FTIR, and GC-MS analyses to determine the number of compounds present, their types of chemical bonds/functional groups, and their full identity and structures, respectively.

Toxicological studies

Acute toxicity studies

The acute toxicity of the Crude aqueous stem bark extract of *Boswellia dalzielii* (CASEB) was evaluated using the up and down procedure of Dixon [25], cited by Abdulazeez et al. [22] with little modification. Ten adult, female, non-pregnant albino Wister rats were selected at random, weighed, and divided into test group (Group A, n = 5 rats) and control group (Group B, n = 5 rats). The two groups were housed in different cages, food was withdrawn for one night, and subsequently each rat in group A was given a single oral dose (3,000 mg/kg body weight) of the extract solution (prepared in sterile distilled water). Group B rats, the negative control, were given normal saline. All the animals were monitored for 48 h for signs of acute toxicity or death instantly.

Sub-chronic toxicity studies

Forty rats were selected at random, weighed, and shared into four groups of 10 rats each. Animals in groups II, III, and IV were, respectively, administered with daily single oral doses of 900, 1,800, and 2,700 mg kg^{-1} body weight of the extract each for 28 days, while those in group I (control) received 2 ml of normal saline daily. A constant dosing time was maintained daily and the doses were adjusted weekly according to the changes in the weights of the animals. The animals were adequately fed, watered, and constantly monitored for signs of morbidity and mortality.

Assessment of liver function

At the terminal stage of the 28-days sub-chronic toxicity study period (28 days), the animals were fasted overnight and euthanized on the 29th day. Blood samples were

collected immediately by cardiac puncture into EDTA and lithium heparin bottles for the biochemical analyses in which an automated determination of serum activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum protein level and, serum bilirubin level were carried out according to standard methods.

Histopathological investigations and microscopic examinations

For the histopathological investigations, selected organs like liver and kidneys were carefully dissected out, removed, and stored in 10% formaldehyde.

The preserved liver and the two kidneys from each rat were subjected to tissue processing procedures for preparation of permanent mount for each tissue as described by Silverto and Baker [26]. The tissues were first dehydrated through various grades of alcohol 30%, 50%, 70%, 90%, and a final bath in 100% alcohol twice to ensure total elimination of moisture. The tissues were cleared in Toluene so as to raise their transparency to refractive index of glass (1.55) to enable transparency of the inclusions. This was followed by the infiltration and embedding of the tissues using liquid paraffin and molten paraffin wax using L-shaped mould, respectively. Sections of the tissues were then made using a Rotary Microtome and the Hot plate method was used for mounting the tissues sections onto the slides. The mounted tissues sections were then stained using Haematoxylin and Eosin stain. The slides were viewed under X-40 objective lens of Microscope and the images were photographed.

Results

Physical characteristics and the percentage yield of the extract

The physical characteristics and the percentage yield of the extract are shown in Table 1. Both the filtrate and residue appeared red in color, whereas the extract appeared dark red in color with solid texture. The percentage yield of the extract was 11.8% of the total weight of the sample extracted.

Table 1. Physical characteristics and the percentage yield of the extract.

S/N	Parameters	Observations
1	Weight of the stem bark powder extracted	3,000.0g
2	Colour of the filtrate	Red
3	Colour and texture of the extract	Dark red and solid
4	Weight of the extract	354.0 gm
5	Percentage yield	11.8%

Preparative thin layer chromatography

The result of Prep TLC of the extract is shown in Table 2. From the result, five fractions were obtained each with different RF values and physical characteristics.

Contact bioautography studies

The result of the direct bioautography test carried out on the separate TLC fractions of the extract against each of the test organisms by disc diffusion method showed that each of the five fractions has antimicrobial activity toward at least one of the test isolates. The most bioactive fraction was Rf2 fraction, which was active against *C. albicans*, *S. typhi*, *S. aureus*, *P. aeruginosa*, *P. mirabilis*, *E. faecalis*, and *S. pyogens* (Table 3).

High performance liquid chromatography of the most bioactive TLC fraction

The HPLC chromatogram of the blank (control) and the most bioactive TLC fraction of the extract is shown in Figure 1a and b, respectively. The chromatogram showed that the bioactive fraction contains seven components. The detailed characteristics of these components are shown in Table 4. From the table, the constituents were at peaks 1, 2, 3, 4, 5, 6, and 7 with corresponding peak areas of 8.57%, 22.25%, 18.14%, 4.40%, 20.41%, 5.99%, and 20.23%, respectively.

Table 2. Preparative TLC showing the different fractions of the aqueous extract partitioned on silica gel plate using chloroform: methanol: water (95:9:1) solvent system.

Rf no.	Rf value	Appearance of the band under UV light at		Weight of the scraped band (mg)	Weight of the extract recovered from the band (mg)
		254 nm	365 nm		
1	0.0278	Brown	Brown	59.35	37.54
2	0.3889	Dark brown	Brown	72.13	19.22
3	0.6667	Light brown	Light blue	92.53	20.51
4	0.8889	Blue	No Absorbance	23.66	8.03
5	0.9444	Blue	Pink	15.32	5.44

Table 3. Antimicrobial activities of the various TLC fractions of the aqueous extract of the stem bark of *B. dalzielii* on the test organisms.

S/N	Test organisms	Various TLC fractions of the aqueous extract of *B. dalzielii*				
		Rf 1	Rf 2*	Rf 3	Rf 4	Rf 5
1	*S. aureus*	–	+	–	–	+
2	*E. faecalis*	–	+	–	–	–
3	*S. pyogenes*	–	+	–	–	–
4	*Ps. aeruginosa*	+	+	+	+	–
5	*K. pneumonia*	+	–	–	–	–
6	*E. coli*	+	–	–	+	–
7	*S. typhi*	+	+	+	–	+
8	*P. mirabilis*	–	+	–	–	–
9	*C. albicans*	–	+	–	+	+

+ = indicates activity; – = indicates no activity; * indicates most bioactive fraction.

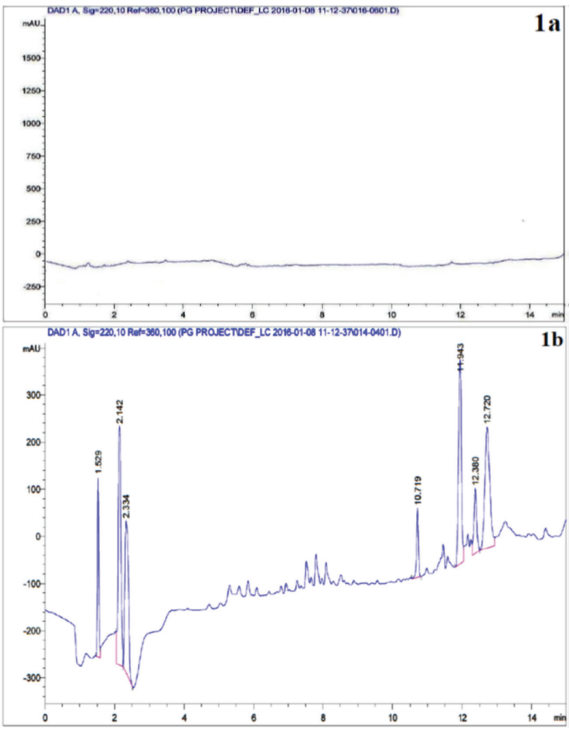


Figure 1. HPLC chromatogram of the blank/distilled water (negative control; 1a) and HPLC chromatogram of the most bioactive TLC fraction (RF 2; 1b) of aqueous extract of stem bark of *Boswellia dalzielii,* respectively.

Fourier transformed infrared spectrophotometry of the most bioactive TLC fraction

The FTIR spectra of the most Bioactive TLC fraction of *B. dazielii* are shown in Figure 2. From the figure, the fraction contained 17 types of bonds/functional groups. The detailed characteristics of these functional groups are shown in Table 5. From the table, the fraction contained 1°, 2° amines and amides, alcohols, phenols, alkanes, alkenes, alkynes, alkyl halides, carboxylic acids, esters, ethers, aromatics, aromatic amines, and aliphatic amines.

Gas chromatography mass spectrometry of the most bioactive TLC fraction of the aqueous stem bark extract of B. daziielii

The total ion chromomatogram (TIC) of the most bioactive fraction of the extract is shown in Figure 3. From the analysis, five compounds have been elucidated and effectively matched and identified. The major constituents were at peaks 5 [(z)- 9-Octadecenoic acid (Oleic Acid) with peak area 44.88%], peak 4[2,6,10,15,19,23 hexamethyl 2,6,10,14,18,22-Tetracosahexaene, (Squalene) with peak area 34.16%], and peak 3 (n-Hexadecanoic acid with peak area 14.14%). While the remaining two compounds constituted less than 8% of the fraction (Table 6).

Acute toxicity study

All animals treated with the oral doses of 3,000 mg/kg body weights of the aqueous extract of the stem bark of *B. dalzielii* exhibited normal behavior and none of them died or showed any sign of acute toxicity during the 48-h observation period. This, therefore, indicates that the median lethal dose (LD_{50}) of the CASEB is greater than 3,000 mg/kg in rats following the up and down toxicity testing procedure.

Sub-chronic toxicity studies

The effects of the aqueous extract of the stem bark of *B. dalzielii* on liver function parameters are presented in Table 7. The result generally indicated a significant ($p < 0.05$) dose-dependent elevation in liver function indices. In

Table 4. Phytochemical components of the RF2 TLC fraction (bioactive fraction) of aqueous extract of the stem bark of *Boswellia dalzielii* obtained by HPLC analysis.

Peak no.	Retention time (min)	Area (%)
1	1.529	8.57
2	2.142	22.25
3	2.334	18.14
4	10.719	4.40
5	11.943	20.41
6	12.380	5.99
7	12.72	20.23

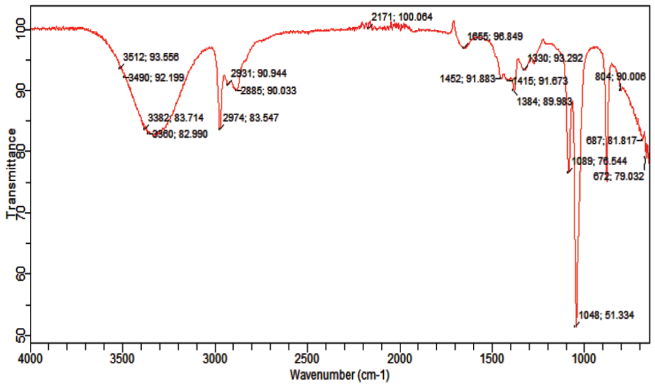


Figure 2. FTIR spectra of most bioactive TLC fraction (RF 2) of aqueous extract of stem bark of *Boswellia dalzielii.*

other words, higher concentrations of the various extract seem to influence the liver function parameters.

Similarly, the histopathology of the liver of the control groups and the groups treated with 900 mg/kg showed normal histology with sheets of hepatocytes having round to oval nuclei with moderate eosinophilic cytoplasm (Fig. 4A and B). The sections of liver of rats treated with 1,800 mg/kg body weights, on the other hand, showed fatty change (Fig. 4C). Several mature adipocytes were seen. The cells had eccentrically placed round to oval nuclei with clear abundant cytoplasm. A centrally placed dilated vein was also noted with few unremarkable hepatocytes. Similarly, sections of the liver of rats treated with the highest concentrations of the extract (2,700 mg/kg) also showed fatty changes (Fig. 4D) with mild lympocytic infiltration, sign of acute hepatitis.

However, the result of the Histopathological investigations of the kidneys of both the control groups and the various groups treated with the extracts showed normal histology (Fig. 4E and F). The cortex demonstrated several glomeruli and the medulla showed many remarkable tubules. In other words, the kidneys of all the groups treated and control were essentially normal.

Discussion

Plants are known to synthesize and accumulate various chemical compounds (phytochemicals) as their secondary metabolites which are mainly used by the plants for defensive purposes. Antimicrobial compounds are amply available in medicinal plants as documented previously [1]. The relative solubility of these compounds in various solvents depends on the nature of chemical bonds and type of functional groups in them as well as the polarity of the solvent used. The result of the percentage yield of the extract (11.8%) in this study showed that water is not a preferable solvent for the extraction of phytochemicals in stem bark of *B. dalzielii* because a similar study [19] showed that a lesser polar solvent (ethanolic solvent) extracted more phytochemicals (13.4%) from the stem bark of the plant. This is in line with the report of Kordali et al. [27] who worked on *Cassia* spp. and *Pistia* spp., respectively, and found that the percentage of extract recovered was defendant on the polarity of the solvent used for the extraction.

Preparative TLC remains an important tool for the isolation and purification of plant extracts [28] and investigation of their biological activity [29]. Compounds are separated based on their polarity and retention indexes, which makes them to form various bands along the TLC plates that could be visualized under UV light, as different compounds have different absorption characteristics. The result of prep TLC in this study produced five bands of different colors under UV light. This suggests that the compounds in the five bands were not the same. Similarly, the fact that Rf 4 band of the extract showed no absorbance under UV light at 365 nm further suggests that compounds exhibit different absorption characteristics at different wavelength of UV light.

The result of contact bioautography indicated that *B. dalzielii* contained various types of antimicrobially active compounds because all the TLC fractions possess antimicrobial activity. The fact that Rf 2 band was the most bioactive indicates that it contains most of the compounds responsible for the earlier reports [12,19,22] of antimicrobial activity of the stem bark the plant. This was further confirmed by the result of HPLC, FTIR, and GC-MS analyses that revealed the identity of the compounds in the band to be Oleic acid (44.88%), which is the major component of unsaturated fatty acid found in Olive oil that is responsible for its antimicrobial, antioxidant, and anticancer activities [30]; Squalene (34.16%), which is the main component of shark oil that is responsible for its strong antioxidant, anti-aging, antimicrobial, anti-cancer, and anti-hypocholostromic properties [31]; and n-Hexadecanoic acid (14.14%), which has been shown to have nematicide, pesticide, lubricant, anti-androgenic,

Table 5. Various functional groups/chemical bonds present in the RF2 (most bioactive TLC fraction) of aqueous extract of the stem bark of *B. dalzielii* Identified by FTIR analysis.

Peak no.	Wave length (cm^{-1})	Transmittance (*t*)	Peak shape	Type of bond identified	Functional group
1	3512	93.556	Narrow	O–H stretch, H–bonded	alcohols, phenols
2	3,490	92.199	Narrow	O–H stretch, H–bonded	alcohols, phenols
3	3,382	83.714	Narrow	N–H stretch	1°, 2° amines, amides
4	3,860	82.99	Curve	O–H stretch, free hydroxyl	alcohols, phenols
5	2,974	83.547	Sharp	C–H stretch	Alkanes
6	2,931	90.944	Narrow	C–H stretch	Alkanes
7	2,885	90.033	Curve	C–H stretch	Alkanes
8	2,171	100.064	Weak	–C=_C– stretch	Alkynes
9	1,655	96.849	Curve	–C=C– stretch	Alkenes
10	1,452	91.883	Narrow	C–C stretch (in–ring)	Aromatics
11	1,415	91.673	Narrow	C–C stretch (in–ring)	Aromatics
12	1,330	93.292	Narrow	C–O stretch	alcohols, carboxylic acids, esters, ethers
13	1,089	76.544	Sharp	C–N stretch	Aliphatic amines
14	1,048	51.334	Very sharp	C–N stretch	Aliphatic amines
15	804	90.006	Narrow	C–Cl stretch	Alkyl helides
16	687	81.817	Weak	C–Br stretch	Alkyl helides
17	672	79.032	Weak	C–Br stretch	Alkyl helides

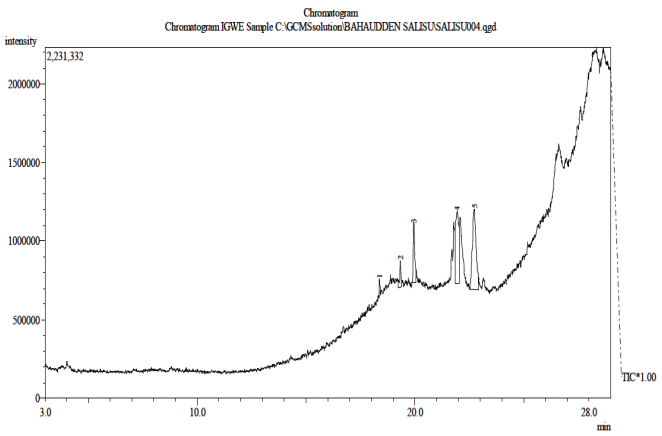


Figure 3. GC-MS showing TIC of most bioactive TLC fraction (RF 2) of aqueous extract of stem bark of *Boswellia dalzielii.*

flavor, hemolytic 5- alpha reductase inhibitor, antioxidant, and hypo-cholesterolemic properties [32].

On the other hand, the result of toxicity studies showed that the LD_{50} was greater than 3,000 mg/kg. This indicates that oral intake of the plant within short period had no toxic effect on rats. However, in the sub-chronic toxicity study where a 28-day study was considered, since it is fully affirmed to evoke several toxicities on long term exposure [33], there were significant changes ($p < 0.05$) in the liver function indexes.

Enzymes are generally involved in virtually all the metabolic activities of the body and hence the assessment of their level of activity in body tissues and fluids is being used as an important tool in the diagnosis of diseases [34]. Enzymes such as ALT, ALP, and AST are generally used for the assessment of liver functions [35]. In the present study, there was a significant ($p < 0.05$) dose-dependent increase in the levels of ALT, ALP, and AST in the serum of rats administered with the extract from 900 to 2,700 mg/kg. Abdulazeez et al. [22] obtained a similar increase in these enzymes activity in serum samples of rat models pre-treated with the aqueous stem bark extract of *B. dalzielii* and has associated the changes to the damages in the tissues where the enzymes are found. According to Cheesbrough [24], the levels of serum ALT and AST are always found to increase when there is liver cell destruction and the higher the level of damage, the higher will be the activities of these enzymes. On the other hand, ALP is well known for its significant role as a marker enzyme for the plasma membranes and endoplasmic reticulum. It is commonly applied to determine the integrity of plasma membrane [36,37]. Thus, the observed dose-dependent increased in the level of activity of this enzyme could be traced to possible damage of hepatocytes plasma membranes that led to its leakage into the blood stream.

Table 6. Bioactive phytochemical compounds identified form the Rf 2 of TLC fraction of the aqueous extract of the stem bark of *Boswellia dazielii* by GC-MS analysis.

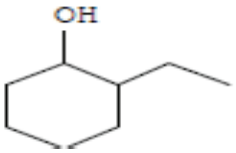
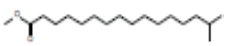
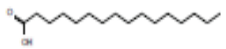
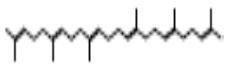
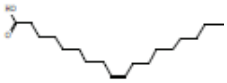
Peak number	Retention time (min)	% composition by area	Matched compound IUPAC name	Structure
1	18.375	1.80	2-Ethylcyclohexanol	
2	19.331	5.02	15-methyl-Hexadecanoic acid	
3	19.950	14.14	n-Hexadecanoic acid	
4	21.942	34.16	2,6,10,15,19,23-hexamethyl-2,6,10,14,18, 22-Tetracosahexaene, (Squalene)	
5	22.727	44.88	(z)- 9-Octadecenoic acid (Oleic Acid)	

Table 7. Mean and standard error of mean values of serum liver function indices in rats administered with aqueous stem bark extract of *Boswellia dalzielii*.

Dose (mg kg^{-1})	AST (UL^{-1})	ALT (UL^{-1})	ALP (UL^{-1})	TP (g dl^{-1})	TB (mg dl^{-1})
Control	93.80 ± 0.80	37.00 ± 0.87	49.76 ± 0.28	2.99 ± 0.41	0.09 ± 0.02
900	98.76 ± 0.43*	39.68 ± 0.27 *	45.76 ± 0.05	3.06 ± 0.41*	0.27 ± 0.006*
1,800	103.91 ± 0.43*	43.85 ± 0.90*	65.38 ± 0.25*	3.92 ± 0.46*	0.99 ± 0.02*
2,700	120.04 ± 0.47*	47.07 ± 1.21*	66.59 ± 0.50*	3.96 ± 0.32*	2.17 ± 0.02*

* = Significantly different from control ($p < 0.05$), $n = 3$; AST = aspartate aminotransaminase; ALT = alanine aminotransaminase; ALP = alkaline phosphatase; TP = total protein; TB = total bilirubin.

Bilirubin is generally a byproduct that results from the rapture of red blood cells. The liver excretes bilirubin and is thus a good indicator of liver function [38,39]. Total bilirubin level increased significantly in groups that received the higher doses of the extract (900–2,700 mg/kg). The presence of serum bilirubin is usually due to functional or mechanical destruction in biliary excretion and is detected in numerous cases of acute hepatitis and cholestasis [40,41]. Therefore, the observed increments of TB level in this study further suggest liver dysfunction.

The study also showed that there was a significant ($p < 0.05$) increase in the total protein levels of groups treated with higher doses of the extracts (1,800 and 2,700 mg/kg). According to Emerson et al. [42], an increase in the serum level of protein is a manifestation of tissue injury and indication of liver damage. However, it could also be due to exposure to any environmental stress that could lead to an elevation in the production of stress proteins in the body [43].

The above dose-dependent increase in liver function parameters suggests possible liver toxicity associated with higher doses of the extract taken for a long period. Histopathological investigations of the liver tissues confirmed this, as all the livers of rats treated with 1,800 and 2,700 mg/kg showed significant fatty changes and signs of acute hepatitis. This was further supported by the presence of potentially toxic compounds, 15-methyl-Hexadecanoic acid and 2-ethylcyclohexanol, in the GC-MS analysis of the TLC fraction of the extract [44]. Furthermore, Salisu et al. [39] have also identified some potentially toxic compounds in the ethanolic extract of the stem bark of the plant. However, due to the fact that these toxic compounds are available in trace amount, the extract could only elicit toxicity when consumed in larger quantities over a long period as observed in this study.

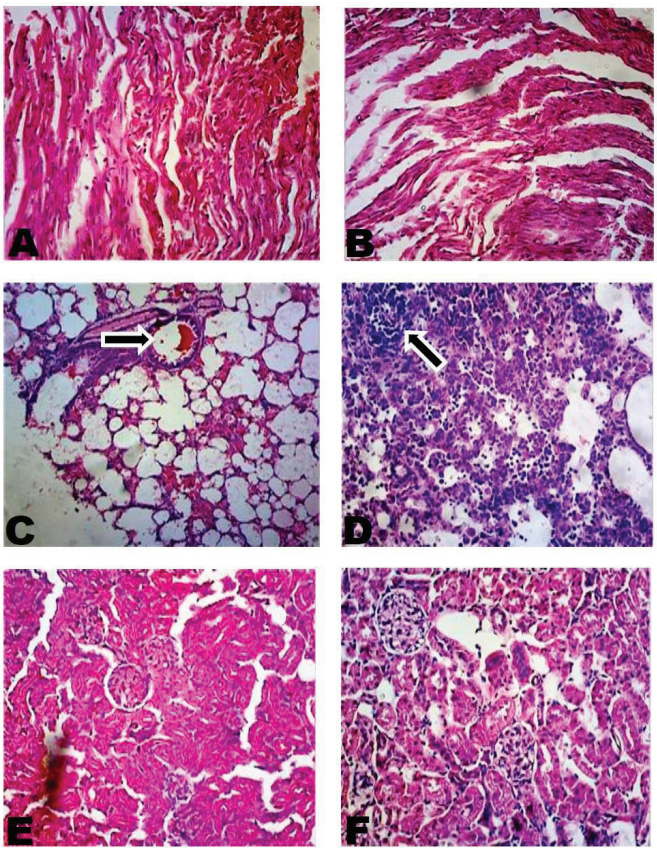


Figure 4. (A) Micrograph of liver of the control groups showing normal histology. Mg = ×400; (B) Micrograph of liver of the group treated with 900 mg/kg showing normal histology. Mg = ×400; (C) Micrograph of liver of the group treated with 1,800 mg/kg showing fatty change and dilated central vain indicated with arrow. Mg = ×400; (D) Micrograph of liver of the Group treated with 2,700 mg/kg showing fatty change and lymphocytic infiltration (Sign of acute hepatitis) indicated with arrow. Mg = ×400, (E) Micrograph of Kidney of the control groups showing normal histology. Mg = ×400; and (F) Micrograph of Kidney of the Group treated with the highest concentration of the extract (2,700 mg/kg) showing normal histology. Mg = ×400.

The histopathology of the kidneys of rats in all the groups, on the other hand showed normal histology. This indicates that the aqueous stem bark extract of *B. dalzielii* is not associated with renal toxicity/damages.

Conclusion

The major antimicrobially active compounds identified from the crude aqueous stem bark extract of *B. dalzielii* in this study were (z)- 9-Octadecenoic acid (Oleic acid), 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-Tetracosahexaene (Squalene), and n-Hexadecanoic acid with percentages of 44.88%, 34.16%, and 14.14% respectively. These compounds could be purified and used for their potentials as candidates of new antimicrobial agent for treating infections due to fungi and *Pseudomonas* spp that are known to resist wide array of antibiotics. However, the study also showed that the oral consumption of the extract over a long duration and at higher dose level should be taken with caution, as it is associated with significant hepatotoxicity.

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Conflict of interests

The authors declared no conflict of interest.

Authors' contribution

Bahauddeen Salisu Dandashire carried out the research under Abdulkadir Magaji Magashi supervision, Bahauddeen Salisu Dandashire and Bashir Abdulkadir conceived and drafted the manuscript, and Muhammad Adamu Abbas, Abdulmalik Yakubu, and Mohammed Dauda Goni reviewed the manuscript. All the authors finally approved the final version for publication.

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