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ABSTRACT

This study was conducted to identify phytochemical constituents present in the methanolic root-bark extract of B. dalzielii using GC-MS analysis and conventional phytochemical evaluation. The studies revealed the presence of alkaloids, flavonoids, saponins, cardiac glycosides, terpenoids and carbohydrates. A total of forty three compounds composed of eighteen major components were oxetane-2,3,4-trimethyl (3.45%), Bicyclo[4.1.0] heptan-3-one-7,7-dimethyl-4identified thus; 2-methyl-2-(-2-oxopropyl) [2.69%], furan (3.20%),Cyclohexane, methylene(IR) (dimethylhydrazono)-3-[4-hexenyl]-1-acinitro,(E,) [2.63%], 3,8-Dihydroxy-3,4-dihydronaphthalen-2-Tetradocanol (4.89%), 3-methyl-2-(2-oxopropyl)furan (3.46%), (2H)-1(2H)one (4.43%), Naphthalenenone, 6-(1,1-dimethyl ethyl) octahydro-2,8a-dimethyl (3.80%), Pentadecanoic acid (6.18%), undecanoic acid (3.68%), Phthalic acid (4.14%), 1-Allyl-cyclohexane-1,2-diol (3.95%), 1,5,9cyclotetradecatriene, 1,5,9-trimethyl-12-(1-methyl ethyl) [4.58%], 9,12-octadecadienoic acid (Z,Z)methyl ester (4.75%). 9-octadecenoic acid, methyl ester[E] (4.36%), Tetradecanoic acid-methyl ester(S) [3.90%], (2S,4R)-p-mentha-[1(7),8]-diene-2-hydroperoxide (2.62%) and 1-Naphthalenepropanol, αethyldecahydro-8a-(hydroxymethyl)-a, 5-dimethyl-2-methylene (2.93%). Most of the identified compounds have been reported to possess various biological activities such as antimicrobial, antiinflammatory, antimalarial, antidiabetics, anti-asthmatic, anti-histamine, antitubercular, antidiarrhoeal among others. The studies thus concluded that B. dalzielii root-bark possesses potent bioactive compounds and is recommended as a plant of phytopharmaceutical importance. The presence of these bioactive compounds justifies the use of this plant in ethnomedicine.

Keywords: Phytochemistry, GC-MS Analysis, Bioactive compounds, Root-bark extract, Boswellia dalzielii

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1. INTRODUCTION

Medicinal plants have been a source of medicinal agents since time immemorial and remarkable number of modern drugs have been derived from natural sources [1]. Natural products especially medicinal plants will continue to play an important role in meeting demand through the expanded investigation of the world's biodiversity, much of which remains unexplored [2]. B. dalzielii is one of the emerging plants of interest in the management of various disease conditions in Nigeria. It is commonly known frankincense tree that belongs to Burseraceae family. It Is popularly known as ararabi and mofu in Hausa and Margi languages in northern Nigeria [3]. The plant demonstrated leadership ethnomedicinal properties in the treatment, prevention and management of different infectious and chronic disease conditions across the globe [1,4]. Traditionally, a decoction of the bark is drunk as a protection against dysentery, hemorrhage and angina [5].

The stem-bark is used in combination with other herbs to treat malaria, yellow fever, stomach ailments, and many childhood diseases [5,6]. The bark is also used to treat gastrointestinal rheumatism, disorders, wounds, asthma, pleurisy, appendicitis, dizziness, palpitation, leprosy, diarrhoea and bloating in cattle [3,4,7,8]. It has antiseptic, wound healing and antifungal potential and is used externally to treat sores, ulcers and dental caries [6]. The root is used as protection against syphilis. The mixture of the roots and bark is a real antidote to poisons and snakebites [6,9]. The decocted root bark is used traditionally by the Hausa-Fulanis in Sokoto, Nigeria to treat diabetes [10]. A bark-decoction is used as an antiseptic wash for sores in Ivory Coast and is an ingredient of a complicated prescription for leprosy [11]. In northern part of Nigeria, the stem bark is boiled and taken for the treatment of fever, rheumatism etc., and the fluid is taken internally gastrointestinal troubles [8,9]. The Fulanis of northern Nigeria uses a cold infusion of the stem bark for the management of snake bite [9]. The fresh bark of the root is eaten in Adamawa State, Nigeria, to relieve symptoms of giddiness and palpitations as well as an antidote of arrow-poison [13,14] amongst other numerous medicinal uses. The plant has shown strong antimicrobial properties in our recent study [1] as well as remarkable antidiabetic effect [4].

This study is designed in order to investigate the major and minor bioactive components of methanolic root-bark extract of *B. dalzielii* using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. GC-MS is one of the best techniques to identify the bioactive constituents of long chain, branched chain hydrocarbons, alcohols, acids, ester, steroids, phenolic compounds, etc. [3,12].

2. MATERIALS AND METHODS

Sample Collection, Identification and Preparation

The sample (*B. dalzielli* root-bark) was collected from Giade local government area of Bauchi state and was identified by a Botanist from the Department of Biological Science University of Maiduguri. The plant material was air-dried at room temperature, grounded with a mortar and pestle and then kept for further analysis.

Sample Extraction

One hundred and seventeen gram of dried powdered material was extracted with absolute methanol using reflux method. After sample has been extracted, the solution was cooled and filtered to remove the debris with filter paper and muslin cloth, and concentrated on hot air oven at 40°C – 50°C to dryness. The extract was weighed and kept in an air tight container for further analysis.

Phytochemical Tests

The qualitative phytochemical screening of the crude extract to identify secondary metabolites such as alkaloids, tannins, flavonoids, terpenes, saponins, cardiac glycosides, anthraquinones, reducing sugars were conducted using standard procedures as described by [13,14,15,16,17,18].

Test for Alkaloids

The extract (0.5g) was dissolved in 10ml of distilled water and the solution was filtered. The solution was then made alkaline using ammonia solution. The mixture was then shaken with two successive portions of chloroform. The combined chloroform layer

was concentrated in vacuo. The residue was reconstituted with 2% sulphuric acid. The resultant acid extract was then tested for the presence of alkaloid by adding a few drops of Dragendorff's reagent to a portion, of the acid extract. The presence of orange precipitate was taken as an indication of the presence of alkaloids. To another portion was added Mayer's reagent and a white precipitate was taken as an indication of the presence of alkaloids [13].

Test for Flavonoids

The extract (0.5g) was dissolved in 10ml of distilled water. To 1ml of the solution was added 10% lead acetate. Yellow gelatinous was taken to indicate the presence of a phenolic nucleus.

To 2ml of the extract in solution (a) above, was added 5 drops of 10% ferric chloride solution. A blue-black coloration indicates the presence of phenolic nucleus. To 2ml of the extract solution in (a) above was added 2ml of 20% sodium hydroxide. A yellow gelatinous precipitate confirms the presence of flavonoids [15].

Test for Saponins Frothing test:

Each extract (5ml) aliquot was placed in a test tube and shaken vigorously. The presence of frothing and time taken (5 minutes) for the froth to disappear was taken as an indication for the presence of saponin [16].

Test for Reducing Sugar Molisch's test:

Each extract (0.5g) was dissolved in water, 2 drops of 10% naphthol were added to 2ml of the dissolved extract, 2ml of concentrated sulphuric acid was added gently down the side of the test tube. A deep violet coloration at the interface indicates the presence of sugars [14].

Fehling Test

The aqueous layer of the extract (5ml) was added into 5ml of Fehling's solution and boiled for 5 minutes. Appearance of a brick red precipitate was taken as an indication for the presence of reducing sugars [14].

Test for Steroids Salkowski's test:

The extract (0.5g) was admixed in 2ml of chloroform. Sulphuric acid was added and the appearance of a reddish – brown colour at the interface indicates the presence of steroidal nucleus [17].

Liberman's test

Five milligrams (500mg) of the extract were dissolved in 2ml acetic anhydride and cooled. Sulphuric acid was then carefully added. A colour change from violet to blue indicated the presence of a steroidal nucleus [18].

Test for Cardiac Glycosides Keller Kelliani's Test:

The extract (0.5g) was dissolved in 2ml of glacial acetic acid containing few drops of ferric chloride solution. This was then underplayed with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicates the presence of deoxy sugar characteristics of cardenolides [14].

Test for Anthraquinone Glycosides Free anthraquinone glycoside

Five milligrams (5mg) of the extract were extracted with hot water for 5 minutes, filtered hot, cooled and extracted with chloroform; 5ml of 10% ammonia solution was added to the separated chloroform. No pink coloration in the ammoniacal phase was observed, indicated the absence of free anthraquinone glycosides [14].

Combined anthraquinones

The aqueous layer, (10 ml) from above was heated with 5ml of 10% sulphuric acid filtered while hot. The filtrate was shaken with chloroform. 5ml of 10% ammonia solution was added to the filtrate. No pink coloration in the ammoniacal layer was observed and this indicates the absence of combined anthraquinone glycosides [14].

Test for Tannins Test for hydrolysable tannins

The extract (0.5g) was boiled in 10ml of distilled water for 5 minutes and filtered. The filtrate was made up to 10ml with distilled water. To 2ml of the filtrate were added 10ml of distilled water and 2 drops of 10% Ferric chloride solution. A blue black was taken as evidence for the presence of hydrolysable tannins [14].

Condensed tannins

To filtrate from above, a drop of bromine water was added and an orange precipitate was taken as evidence of the presence of condensed tannins [14].

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

GC-MS analysis were carried out on a GC-MS-QP 2010 Plus Shimadzu system and Gas chromatograph interfaced to spectrometer (GC-MS) instrument employing the following conditions: 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 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µl was employed (Split ratio of 10:1) injector temperature-250°C; Ion source temperature 280°C. The oven temperature was programmed from 110°C (Isothermal for 2 min.) with an increase of 10°C / min to 200°C then 5C / min. to 280°C / min, ending with a 9 min. isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 40 to 550Da. Total GC running time was 36 minutes. The relative percentage amount of each component was calculated, by comparing its average peak area to the total areas, Software adopted to handle mass spectra and chromatogram was a turbomass. The detection employed was the NIST Ver. 2.0-year 2009 library [3].

Identification of Components

Interpretation of mass spectrum of GC-MS was done using the database of National Institute of Standard and Technology NIST-08 LIB and WILEY-8 LIB. Library sources were used for matching the identified compounds from the plant material having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the unknown components stored in the NIST library [19].

3. RESULTS AND DISCUSSION

Plants are rich source of secondary metabolites with interesting biological activities. There is growing awareness in correlating the phytochemical constituents of medicinal plant with its pharmacological activity [4]. Phytochemical analysis conducted on the

plant extract revealed the presence of constituents which are known to exhibit medicinal as well as physiological activity.

The results of extraction profile presented in (Table 1) showed percentage yield of 16.23% and this indicates that methanol is a good solvent for extraction. Phytochemical analysis of the methanolic root-bark extract of B. dalzielii showed the presence of flavonoids, steroids, cardiac glycosides, tannins, terpenoids, saponins and carbohydrates (Table 2) which is also in agreement with the previous studies carried out by [5,8,11]. GC-MS is one of the best techniques to identify the constituents of volatile matter, long chain, branched chain, alcohols, acids, esters etc. The GC-MS Chromatogram (Fig. 1) showed different peaks indicating various components identified from the methanolic root-bark extract of the B. dalzielii. From the results obtained by GC-MS analysis, a total of fortythree components composed of eighteen (18) major components were detected (Table 3). The percentage of total identified compounds in root-bark extract of *B. dalzielii* is 91.81%. The major compounds detected in this extract are oxetane-2,3,4-trimethyl (3.45%), Bicyclo [4.1.0] heptan-3-one-7,7-dimethyl-4-methylene 2-methyl-2-(-2-oxopropyl)furan (3.20%), cyclohexane, 2-(dimethylhydrazono)-3-[4-hexenyl]-1-acinitro,(E,) [2.63%], Dihydroxy-3,4-dihydronaphthalen-1(2H)one (4.43%), 2-Tetradocanol (4.89%), 3-methyl-2-(2oxopropyl) furan (3.46%),(2H)-Naphthalenenone, 6-(1,1-dimethyl ethyl) octahydro-2,8a-dimethyl (3.80%),Pentadecanoic acid(6.18%), undecanoic acid (3.68%),Phthalic acid (4.14%),1-Allylcyclohexane-1,2-diol (3.95%),1,5,9cyclotetradecatriene, 1,5,9-trimethyl-12-(1methyl ethyl) [4.58%], 9,12-octadecadienoic acid (Z,Z)-methyl ester (4.75%). 9-octadecenoic acid, methyl ester[E] (4.36%), Tetradecanoic acid-methyl ester(S) [3.90%], (2S,4R)-p-[1(7),8]-diene-2-hydroperoxide mentha-1-Naphthalenepropanol, (2.62%) and ethyldecahydro-8a-(hydroxymethyl)-a,5dimethyl-2-methylene (2.93%).

Most of these major phytoconstituents detected from this plant extract have shown a remarkable bioactivity. For instance, oxetane-2,3,4-trimethyl, Bicyclo [4.1.0] heptan-3-one-7,7-dimethyl-4-methylene(IR), 3,8-Dihydroxy-3, 4-dihydronaphthalen-1(2H)one, 3-methyl-2-

(-2-oxopropyl) furan, undecanoic acid and Tetradecanoic acid have been reported to possessed antimicrobial properties [1,20,21,22,23,24,25,26,27]. Other important bioactive components detected that have anti-inflammatory properties include cyclohexanol, 2-Tetradecanol and 1-Naphthalenepropanol, α-ethyldecahydro-8a- (hydroxymethyl)-α, 5-dimethy l-2-methylene [28]. According to the previous reports, [2H]-Naphthalene-one,6-(1,1-dimethyl ethyl) octahydro-2,8a-dimethyl has demonstrated an antidiarrhoeal activity [31] while pentadecanoic acid and 2-methyl-2-

(2-oxopropyl) furan have shown a tremendous antidiabetic activity [28,29,30]. Others include 9,12-octadecamethylester (E) as antihistamine [32,33], phthalic acid (anti-asthmatic) [28] and imidazo [4,5-d] imidazole-1,6-dihydro (antitubercular) [29]. The presence of these bioactive compounds justifies the uses of this plant in ethnomedicine. This study thus concludes that *B. dalzielii* root-bark possess potent bioactive compounds and is recommended as a plant of pharmaceutical importance.

Table 1: Extraction profile of the crude methanolic root-bark extract of *B. dalzielii*

Parameters	Results
Weight(g)	117
% yield	16.23%
Colour	Dark Black
Texture	Amorphous powder

Table 2: Results of phytochemical screening of methanolic root-bark extract of B. dalzielii

S. No.	Test	Result
1	Test for carbohydrates	
	Molisch's Test	+
	Test for monosaccharide (Barfoed's Test)	+
	Test for combined reducing sugar	+
	Test for free reducing sugar (Fehling's Test)	+
	Test for ketoses	+
2.	Test for Alkaloids	
	Dragendroff's reagent	+
	Mayer's reagent	+
3.	Test for Flavonoids	
	Shinoda's test	+
	Ferric Chloride test	+
	Lead Acetate test	+
	Sodium hydroxide	-
4.	Test for saponins	
	Frothing Test	+
5.	Test for steroids	
	Liebermann-Burchard Test	+
	Salkowski's Test	+
6.	Test for anthraquinones	
	Free Anthraquinones	-
	Combined Anthraquinone	-
7.	Test for Cardiac glycosides	
	Keller killiani	+
8.	Test for Tannins	
	Ferric Chloride Test	+
	Lead Acetate Test	+
9.	Test for terpenoids	+

Key: (+) = Present, (-) = Absent

Table 3: Compounds identified in methanolic root-bark extract of Boswellia dalzielii and their bioactivities

Peak No	RT	Name of the compound	% Peak	MF	MW	Structure of the compound	Bioactivity
1	1.188	Silane, dimethyl	Area 15.83	C ₂ H ₈ Si	60	SiH ₂	Anti -malaria
2	1.943	Oxetane, 2,3,4-trimethyl	39.59	C ₆ H ₁₂ O	100	•	Anti-bacterial
3	1.971	2-Pentenenitrile	10.78	C ₅ H ₇ N	81		Anti-bacterial
4	3.316	Butanimidamide, N-(1-chloro-2-methyl-1-butenyl)-2-methyl-	8.77	C ₁₀ H ₁₉ CIN ₂	202	CI NH	Anti-fungal
5	3.803	Imidazo[4,5-d]imidazole, 1,6-dihydro	10.77	C ₄ H ₄ N ₄	108	H H N N N N N N N N N N N N N N N N N N	Anti-tubercular
6	4.512	Bicyclo[4.1.0]heptan-3-one, 7,7-dimethyl-4-methylene-, (1R)-	30.87	C ₁₀ H ₁₄ O	150		Antibacterial
7	4.638	Mequinol	17.72	C ₇ H ₈ O ₂	124	ОН	Antimicrobial
8	4.787	Benzaldehyde, 4-methoxy	24.43	C ₈ H ₈ O ₂	136		Antimicrobial
9	4.981	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-9-(phenylsulfonyl)-, (E,E)-	15.90	C ₂₁ H ₃₀ O ₃ S	362		Antibacterial
10	5.399	2-Methyl-2-(2-oxopropyl)furan	36.76	C ₈ H ₁₀ O ₂	140		Anti-diabetic
11	5.485	Cyclohexane,2- (dimethylhydrazono)-3-[4- hexenyl]-1-aci-nitro-, (E,E)-	30.20	$C_{14}H_{25}N_3O_2$	267		Not Reported

12	5.605	Cyclohexanol, 4-ethyl-4-methyl-3-(1-methylethyl)-, $(1\alpha,3\alpha,4\beta)$ -	26.50	C ₁₂ H ₂₄ O	184		Anti- inflammatory
13	5.862	Bicyclo[3.3.1]nonan-9-ol, 9-methyl-	10.05	C ₁₀ H ₁₈ O	154	OH	Anti- inflammatory
14	6.005	6-(3-Methyl-3-cyclohexenyl)-2- methyl-2,6-heptadienol	18.51	C ₁₅ H ₂₄ O	220	ОН	Antibacterial
15	6.423	3,8-Dihydroxy-3,4- dihydronaphthalen-1(2H)-one	50.82	$C_{10}H_{10}O_3$	178	OH O	Antibacterial
16	6.538	Retinal, 9-cis	28.35	C ₂₀ H ₂₈ O	284	<u> </u>	Anti-diarrhoea
17	6.795	Photocitral	20.62	C ₁₀ H ₁₆ O	152	0	Antibacterial
18	7.116	Cyclobutanone, 2-(2,6-dimethylheptyl)-	14.35	C ₁₃ H ₂₄ O	196		Not Reported
19	7.344	Cyclobutanone, 2-(2,6-dimethylheptyl)-	56.12	C ₁₄ H ₃₀ O	214	ОН	Anti- inflammatory
20	7.625	3-Methyl-2-(-2-oxopropyl)furan	39.72	C ₇ H ₈ O ₂	124	0 -0	Antibacterial
21	7.756	(2H)-Naphthalenone, 6-(1,1-dimethylethyl)octahydro-2,8a-dimethyl-	43.65	C ₁₆ H ₂₈ O	236		Anti-diarrhoea
22	8.054	Pentadecanoic acid, 14-methyl-, methyl ester	70.94	C ₁₇ H ₃₄ O ₂	270		Anti-diabetic

23	8.34	Undecanoic acid	42.27	C ₁₁ H ₂₂ O ₂	186	OH	Anti-fungal
24	8.392	Phthalic acid, isobutyl octadecyl ester	47.51	C ₃₁ H ₄₂ O ₄	478		Anti-asthmatic
25	8.454	1-Allyl-cyclohexane-1,2-diol	45.38	C ₉ H ₁₆ O ₂	156	OH OH	Not Reported
26	8.517	1,5,9-Cyclotetradecatriene, 1,5,9-trimethyl-12-(1-methylethenyl)-	52.60	C ₂₀ H ₃₂	272		Not Reported
27	9.256	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	54.53	C ₁₉ H ₃₄ O ₂	294		Antihistamine
28	9.29	9-Octadecenoic acid, methyl ester, (E)-	49.99	C ₁₈ H ₃₃ O ₂	281		Antihistamine
29	9.479	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	44.75	C ₁₆ H ₃₂ O ₂	256		Antimicrobial
30	9.719	R)-(-)-14-Methyl-8-hexadecyn-1-ol	20.48	C ₁₇ H ₃₂ O	252	ОН	Antimicrobial
31	9.885	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	24.12	C ₂₀ H4 ₀	280		Antimicrobial
32	10.028	(2S,4R)-p-Mentha-[1(7),8]-diene- 2-hydroperoxide	30.09	C ₁₀ H ₁₆ O ₂	168	о-он	Antibacterial
33	10.188 20.95	1-Naphthalenepropanol, α- ethenyldecahydro-8a- (hydroxymethyl)-α,5-dimethyl-2- methylene 1-Cyclohexylnonene	33.64	C ₉ H ₃₂ O ₂	292	HOF	Anti- inflammatory
34	16.545	1-Cyclohexylnonene	9.39	C ₁₅ H ₂₇	207		Antimicrobial

35	18.868	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, $(3\beta,17\beta)$ -	8.70	C ₂₂ H ₃₂ O ₂	328	но	Not Reported
36	9.67	9-Methyl-Z,Z-10,12- hexadecadien-1-ol acetate 2-(3-	8.00	C ₁₉ H ₃₁ O ₂	291		Not Reported
37	20.95	Hydroxybutyl)cyclooctenone	2.77	$C_{12}H_{21}O_2$	198	O OH	Antibacterial
38	28.264	5,9-Hexacosadienoic acid, methyl ester	8.89	C ₂₇ H ₃₀ O ₂	406		Antimicrobial
39	28.853	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl- ,[1aR-1aα,4β,4aβ,7α,7aβ,7bα)]- 2(1H)	8.47	C ₁₅ H ₂₆ O	222	HO	Not Reported
40	30.627	Naphthalenone, 3,5,6,7,8,8a-hexahydro-4,8a-dimethyl-6-(1-methylethenyl)-	5.12	C ₁₅ H ₂₂	218		Not reported
41	32.83	1-formyl-2,2,6-trimethyl-3-(3-methyl-but-enyl)-6-cyclohexene	15.08	C ₁₅ H ₂₄ O	524		Anti- inflammatory
42	35.125	5,9-Hexacosadienoic acid, methyl ester	6.06	C ₂₇ H ₅₀ O ₂	406		Not Reported
43	35.886	8a(2H)-Phenanthrenol, 7- ethenyldodecahydro-1,1,4a,7- tetramethyl-, acetate, [4as- $(4a\alpha,4b\beta,7\beta,8a\alpha,10a\beta)$]-	8.27	C ₂₂ H ₃₆ O ₂	332		Not Reported
Total of identified compounds (%)			91.81%				

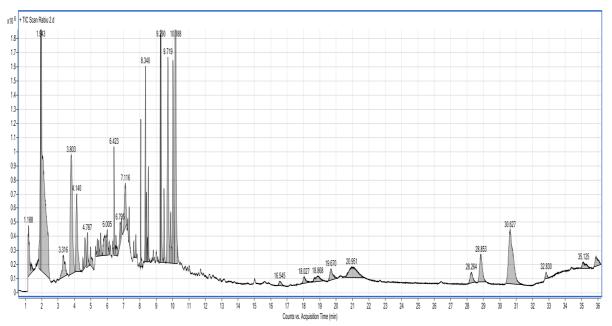


Figure 1: GC-MS chromatogram of methanolic root-bark extract of *B. dalzielii*

4. CONCLUSION

Boswellia dalzielii possesses various bioactive compounds and is recommended as a plant of pharmaceutical importance. Its root-bark can be used as antimicrobial, anti-inflammatory, antidiabetics, antidiarrhoeal, antimalaria, antiasthmatic, antihistamine and antitubercular agents. The presence of the secondary metabolites is responsible for the bioactivities confirmed in this plant. These findings have provided scientific basis to the ethnomedical usage of the plant. Isolation characterization of the active compounds of this important medicinal plant is already on the way.

5. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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