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GC-MS Analysis of Bioactive Compounds from Bark and Leaf of *Boswellia ovalifoliolata*

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The aim of the present study was to identify and characterize the bioactive compounds present in bark and leaf of *Boswellia ovalifoliolata* using GC-MS analysis. A total of 17 fatty acids methyl ester, *i.e*., tetradecanoic acid, 12-methyl-, methyl ester (0.9 %), (s)-, hexadecanoic acid, methyl ester (7.6 %), octadecanoic acid, methyl ester (15.0 %) and triacontanoic acid, methyl ester (6.1 %) was identified and characterized. GC-MS analysis of crude extract showed the presence of 31 compounds, *i.e*., β-amyrin (8.5 %), α-amyrin (29.2 %), stigmasterol (1.5 %), DL-α-tocopherol (7.3 %), β-sitosterol (10.4 %) and vitamin E (2.3 %). This is the first report on the identification of active molecule from different extracts of bark and leaf of *Boswellia ovalifoliolata*. All the major compounds identified in *Boswellia ovalifolilolata* are biologically active molecules.

Keywords: *Boswellia ovalifoliolata*, Fatty acids, GC-MS.

INTRODUCTION

Plants are the important source of chemical diversity for starting material and many chemicals from plants used as pharmaceutical biochemicals, fragrances, food colours and flavours in many countries especially in India [1]. Natural products act as a major source of drugs and also used for the development of novel drugs for the treatment and prevention of many diseases. Most of the medicinal plants and its derivative products were often prepared from crude plant extracts comprise of the complex mixture of different plant secondary metabolite [2-4]. The standardization of crude extracts is an integral part of finding its correct structure and activity. In order to validate the use and activity of the medicinal plant, the chemical composition of the plant should be investigated [5].The preliminary screening of the medicinal plant by spectrometric and chromatographic method provides information on chemical and pharmacological activity of the plant to select the effective crude plant extract [6]. Gas chromatography-mass spectrometry (GC-MS) is a fast and accurate technique to identify the bioactive compound present in the plant extract.

Boswellia ovalifoliolata N.P. Balakr & A.N. Henry belongs to Burseraceae family is an endangered, endemic tree species belongs to Seshachalam hill range of eastern ghats of India. It is commonly known as guggilam, konda sambrani and adavi sambrani by the local tribes of the village. The tribal people like nakkala, sugali, chenchu and indigenous people in nearby villages make a deep cut in the trunk to extract gum which causes damage and depletion of the plant species and is used to treat various ailments. The tribal people used gum powder, leaves, and stem bark to treat dysentery, inflammation, joint pains, ulcers, arthritis and amoebic dysentery. The stem bark of the plant used to treat rheumatic pain and also to reduce pain when administered orally, whereas the leaf part of the plant is used for mouth and stomach ulcers. Boswellic acid and amyrins are the main constituents present in all the species of Boswellia, which make it more distinctive of the genus [7]. Ethanolic extract of oleo gum resin contain 1β,3β,11α-trihydroxy-urs-12-ene in 3α-hydroxytricall-8,24-dien-21-oic acid, 3α-acetoxy-tirucall-8,24-dien-21-oic acid, serratol, neoilexonol, 3-hydroxy-urs-9,11-dien-24-oic acid were firstly reported by Chib *et al*. [8]. There is no literature available on the chemical constituents of different crude extracts of *Boswellia ovalifoliolata*. The main objective of this study is to identify the phytoconstituents present in the various crude extracts and isolation of fatty acids.

EXPERIMENTAL

Analytical grade solvents (hexane, chloroform, ethyl acetate, methanol and diethyl ether were purchased for the experiment. All other chemicals used in the study were of analytical grade. The rotary evaporator (Buchi, Rotary evaporator, model- R 3) was used to concentrate the extract.

Collection of plant: The plant was collected from Seshachalam hills of Andhra Pradesh state of India during the month of February

2015. A voucher specimen (PARC/2015/3202) was deposited at Plant Anatomy Research Centre and was authenticated by Prof. Jayaraman, Presidency College, Chennai, India.

Preparation of *Boswellia ovalifoliolata* extracts: The plant material (leaf and bark) was thoroughly washed with distilled water to remove all the contaminants from the plant. It was shade-dried in room temperature to retain all the phytochemicals and made into fine powder using a blender. The powdered plant material was used for the further extraction. About 10 g of dried plant material was packed in Soxhlet apparatus for the sequential extraction of the plant using a Soxhlet extractor. About (500 mL) solvent were used in increasing order of polarity (hexane, chloroform, ethyl acetate and methanol) to extract phytochemicals of all polarities. The extraction was carried out till all the solvent in the Soxhlet apparatus turns colourless. Further, the extracts were concentrated using a rotary evaporator. It was weighed and stored in a vacuum desiccator. The percentage yield was calculated for all the extracts.

GC-MS analysis: The GC-MS analysis of different crude extracts of *B. Ovalifoliolata* was performed using Perkin Elmer Clarus 680 equipped with Mass Spectrometer Clarus 600 (EI). The Elite-5 MS (30 m, 0.25 mm ID, 250 µm df) column was used for the separation of the compounds from the extracts. The oven initial temperature was 60 ºC for 2 min, ramp 10 ºC/min to 300 ºC, hold 6 min, the injector temperature was 250 ºC and the oven temperature was maintained at 300 ºC for 6 min. The carrier gas used was helium with a constant flow rate of 1 mL/min. The mass transfer line and source temperature were set at 240 ºC and 240 ºC, respectively. The identification of the compounds and structure determination were based on the comparison of mass spectra and their fragmentation profiles using published data, Wiley, NIST library search.

Isolation of fatty acids: The petroleum ether solvent was employed for the isolation of fatty acids from *Boswellia ovalifoliolata*. The bark and leaf part of the plant was extracted using petroleum ether to obtain fatty oil and evaporated using rotavapor. The oil (0.4 mg) was hydrolyzed with 0.5 N KOH in ethanol (15 mL) for 4-7 h. The free fatty acids were separated after hydrolysis. The free fatty acids were then extracted with diethyl ether (10 mL) to obtain unsaponified fraction and allowed to stand for a few minutes. The upper layer contains unsaponified part and the lower water layer contains free fatty acids was collected.The water layer (containng free fatty acids) was mixed with HCl to make it acidic and extracted again with diethyl ether for the complete separation of free fatty acids. The ether layer was completely evaporated to dryness at room temperature. About 25 mL of 2 % methanolic sulphuric acid was added to free fatty acids to obtain methyl esters of fatty acids with 6 h heating in water-bath. Diethyl ether and 1 % KOH was added and the further ether layer was separated. The aqueous layer was extracted again with ether for the complete removal of fatty acids. The ether layer was combined washed with distilled water and dried using anhydrous sodium sulphate. The dried fatty acids were dissolved in the solvent for GC-MS analysis [9].

RESULTS AND DISCUSSION

GC-MS analysis: The identified bioactive compounds present in different crude extract of *B. ovalifoliolata* analyzed by using GC-MS are listed in Table-1. The hexane extract of bark showed seven peaks in GC-MS chromatogram (Fig. 1A), five compounds were identified in comparison with NIST library and the major compound was found to be α-amyrin (99.8 %), 4,4,6a,6b,8a,11,11,14-boctamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2*H*-picen-3-one (56.7 %), olean-13(18)-ene (20.1 %), caryophyllene (1.1 %) and (E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentane (0.9 %). The identified compounds in chloroform extract contains

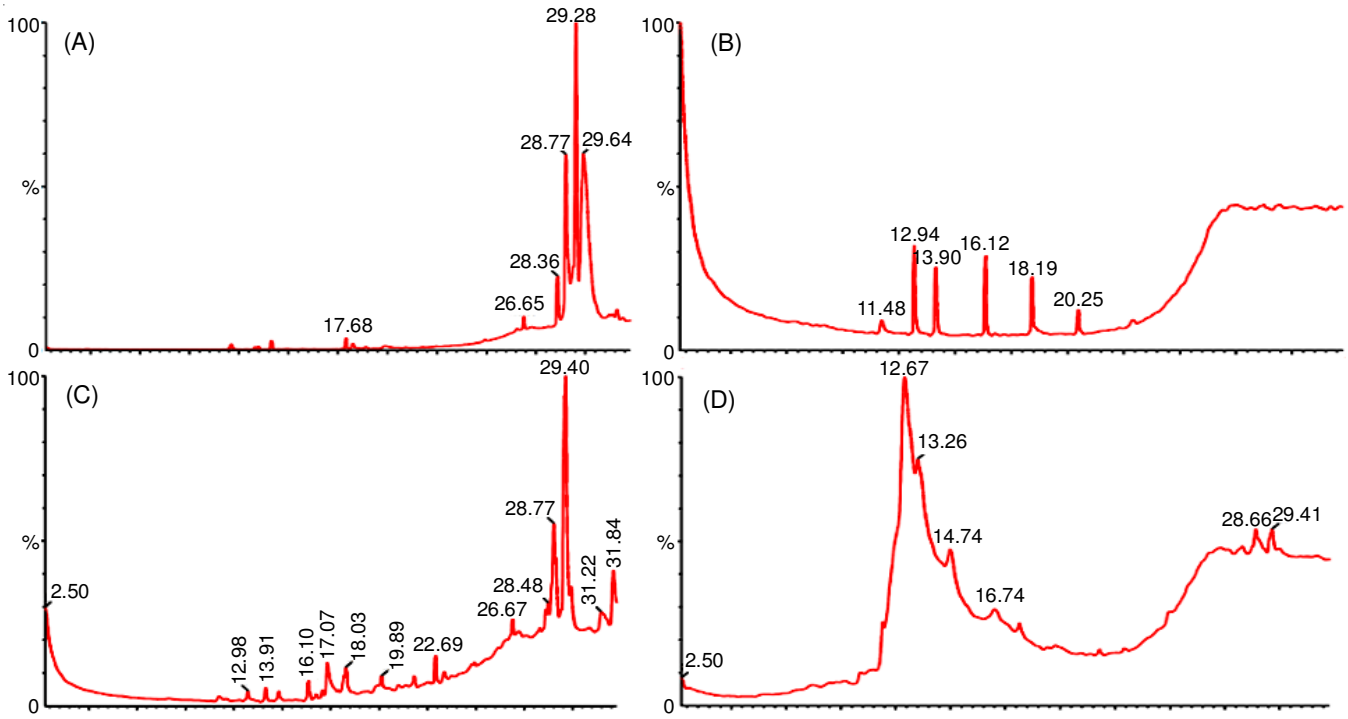

Fig. 1. GC-MS chromatogram of hexane (A), chloroform (B), ethyl acetate (C), methanolic (D) extracts of bark

TABLE-1
CHEMICAL COMPOSITION OF DIFFERENT EXTRACTS OF *B. ovalifoliolata*

Extract	Area (%)	RT	Name	m.f. (m.w.)
Hexane extract (Bark)	1.1	11.85	Caryophyllene	$C_{15}H_{24}$ (204)
	0.9	18.00	(E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene	$C_{20}H_{32}$ (272)
	20.1	28.33	Olean-13(18)-ene	$C_{30}H_{50}$ (410)
	56.7	28.75	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11, 12,12a,14,14a,14b-octadecahydro-2*H*-picen-3-one	$C_{30}H_{48}O$ (424)
	99.8	29.27	α-Amyrin	$C_{30}H_{50}O$ (426)
Chloroform extract	22.2	12.98	Phenol	$C_{14}H_{22}O$ (206)
Ethyl acetate extract	1.1	14.56	Phosphine	
	0.5	16.83	Benzene dicarboxylic acid ester	
	2.2	26.65	Methyl 3-bromo-1-adamantaneacetate	
	10.6	28.77	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11, 12,12a,14,14a,14b-octadecahydro-2*H*-picen-3-one	
Methanol extract	11.89	14.75	2,2-dimethylpropanoic acid, hex-4-yn-3-yl ester	$C_{11}H_{18}O_2$ (182)
Hexane extract (Leaf)	0.5	18.6	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256)
	7.3	27.41	DL-α-tocopherol	$C_{29}H_{50}O_2$ (430)
	20.7	30.09	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11, 12,12a,14,14a,14b-octadecahydro-2*H*-picen-3-one	$C_{30}H_{48}O$ (424)
Chloroform extract	1.5	13.28	Phenol	$C_{14}H_{22}O$ (206)
	1.3	14.25	Hexadecene	$C_{16}H_{32}$ (224)
	0.8	16.23	Tetradecanoic acid	$C_{14}H_{28}O_2$ (228)
	5.2	18.25	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256)
	0.5	18.49	Eicosene	$C_{20}H_{40}$ (280)
	2.3	19.92	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$ (224)
	1.3	26.87	5.Alpha.-Pregnane-12,20-Dione, Cyclic 12-(Ethylene Mercaptole)	$C_{23}H_{36}OS_2$ (392)
	4.0	27.66	DL-α-Tocopherol	$C_{29}H_{50}O_2$ (430)
	1.5	29.02	Stigmasterol	$C_{29}H_{48}O$ (412)
	10.4	29.75	β-Sitosterol	$C_{29}H_{50}O$ (414)
	8.5	30.06	β-Amyrin	$C_{30}H_{50}O$ (426)
	13.14	30.96	α-Amyrin	$C_{30}H_{50}O$ (426)
Ethyl acetate extract	7.0	11.612	1,1,3,3-Tetramethyl-1,3-disilaphenalane	$C_{15}H_{20}Si_2$ (256)
	15.1	13.16	Valeric acid, hex-4-yn-3-yl ester	$C_{11}H_{18}O_2$ (182)
	3.6	17.83	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256)
	2.3	26.89	Vitamin E	$C_{29}H_{50}O_2$ (430)
Methanol extract	32.0	13.31	Phenol, 2-[(1-methylpropyl)	$C_{10}H_{14}O$ (150)

six peaks (Fig. 1B), only one compound was identified as phenol (12.9 %), whereas ethyl acetate extract contains fourteen peaks (Fig. 1C), four compounds were identified 4,4,6a,6b,8a, 11,11,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12, 12a,14,14a,14b-octadecahydro-2*H*-picen-3-one (10.6 %), 4,4,6a,6b,8a,11,11a,14b-octamethyl methyl 3-bromo-1-adamantane acetate (2.2 %), phosphine (1.1%). The polar methanol extract contains only one compound (Fig. 1D) hex-4-yn-3-yl 2,2-dimethylpropanoic acid ester (11.8 %). The GC-MS chromatogram of hexane extract of leaf are shown in (Fig. 2A). The hexane extract of isolated from leaves of *B. ovalifoliolata* analyzed by using GC-MS led to the identification of three compounds with major compounds were found in 4,4,6a,6b,8a,11,12,14b-octamethyl-1,4,4a,5,6, 6a,6b,7,8,8a,9,10,11,12,12a,14,14a, 14b-octadecahydro-2*H*-picen-3-one (30.0%), DL-α-tocopherol (27.4 %), *n*-hexade-canoic acid (18.6 %). The major compounds in chloroform extract was found to be α-amyrin (13.1 %), β-sitosterol (10.4 %), β-amyrin (8.5 %) and *n*-hexadecanoic acid (5.2 %). The GC-MS chromatogram of chloroform extract (Fig. 2B) contains 18 peaks, only 12 compounds were identified. The results revealed the presence of 4 compounds in ethyl acetate extract (Fig. 2C), hex-4-yn-3-yl valeric acid ester (15.1 %), 1,1,3,3-tetramethyl-1,3-disilaphenalane (7.0 %), *n*-hexadecanoic acid (3.6 %) and vitamin E (2.3 %). Finally, the methanolic extract (Fig. 2D) showed 6 peaks and but only one compound was identified 2-[(1-methyl propyl)phenol (32.0 %).

GC-MS analysis of crude extracts (31 compounds) of *B. ovalifoliolata* (Table-1) showed the presence of β-amyrin, α-amyrin, stigmasterol, DL-α-tocopherol, β-sitosterol and vitamin E as the active compound. β-Amyrin and α-amyrin possess to have antimicrobial, antifungal and antiinflammatory activity [10]. Stigmasterol is also called as phytosterol have been reported for the prevention of prostate, ovarian, breast and colon cancer [11]. DL-α-tocopherol reported having antioxidant activity, immune activity, anticancer activity, anti-inflammatory activity, anticoagulant and antiplatelet activity [12]. The major compound from the crude extract is biologically active compound.

Isolation of fatty acids: The separation of fatty acids by petroleum ether extract of *B. ovalifoliolata* are presented in Table-2. The results showed the presence of 4,4,6a,6b,8a,11, 12,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a, 14,14a,14b-octadecahydro-2*H*-picen-3-one (21.1 %), hop-22-(29)-en-3-β-ol (13.1 %), betulin (9.3 %), bicyclo[3.1.0]hexan-2-one, 1,5-*bis*(1,1-dimethylethyl)-3,3-dimethyl (7.7 %), triacontanoic acid, methyl ester (6.1 %), A-neooleana-3(5),12-diene (2.4 %), 3,5-di-*tert*-butyl-4-hydroxy benzaldehyde (1.7%), ursa-9(11),12-dien-3-one (1.4 %), hexadecanoic acid, methyl ester

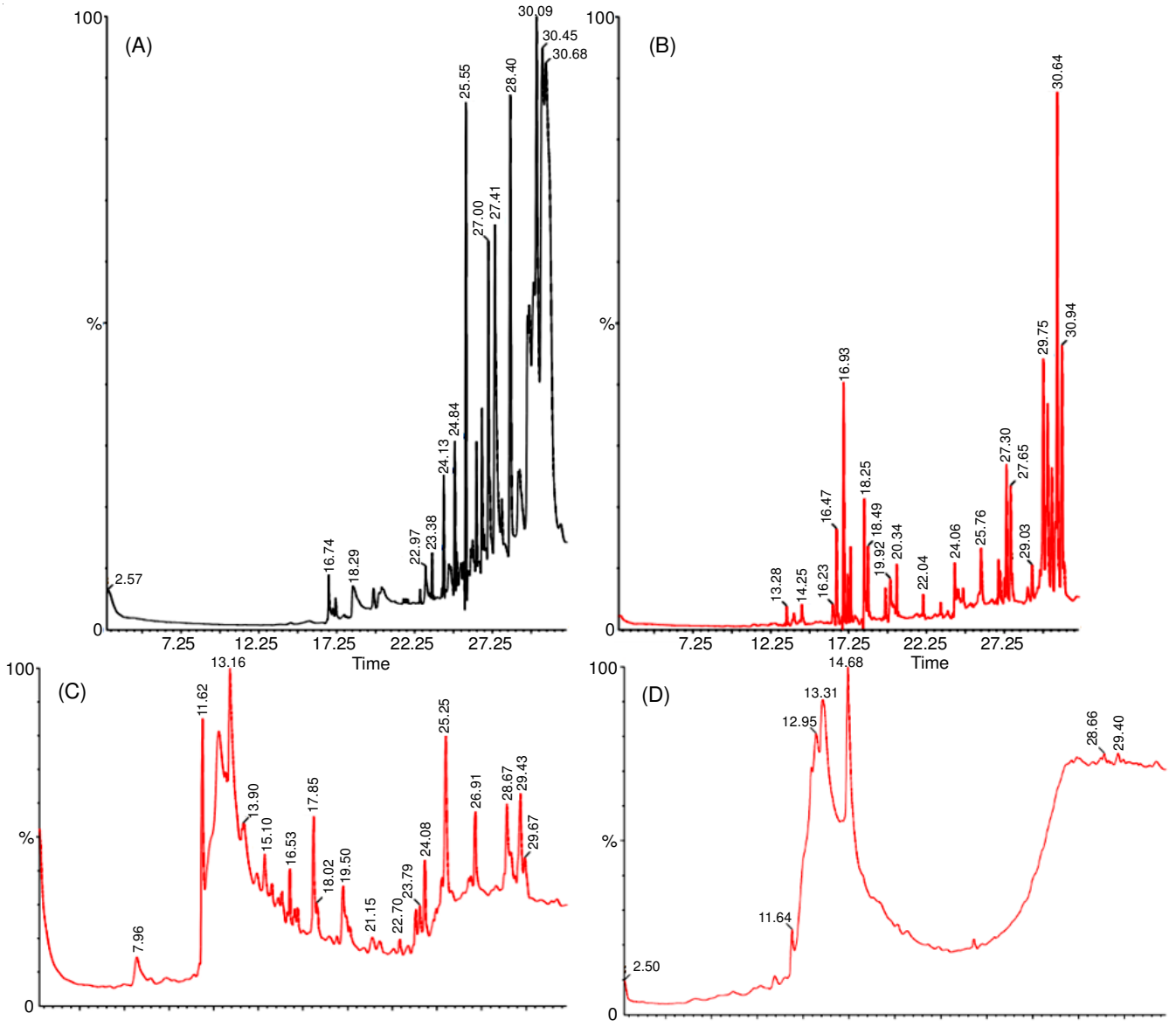

Fig. 2. GC-MS chromatogram of hexane (A), chloroform (B), ethyl acetate (C), methanolic (D) extracts of leaf

TABLE-2
IDENTIFIED FATTY ACID METHYL ESTERS FROM PETROLEUM ETHER EXTRACT OF *B. ovalifoliolata*

RT	Area (%)	Name	m.f. (m.w.)
		Bark	
12.65	7.7	Bicyclo[3.1.0]hexan-2-one, 1,5-*bis*(1,1-dimethylethyl)-3,3-dimethyl	$C_{16}H_{28}O$ (236)
16.30	1.7	3,5-di-*tert*-butyl-4-hydroxybenzaldehyde	$C_{15}H_{22}O_2$ (234)
17.76	1.2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ (270)
19.28	1.1	9-octadecanoic acid (z)-, methyl ester	$C_{19}H_{36}O_2$ (296)
19.46	0.9	Tetradecanoic acid, 12-methyl-, methyl ester, (s)-	$C_{16}H_{32}O_2$ (256)
24.13	6.1	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$ (466)
25.59	2.4	A-neooleana-3(5),12-diene	$C_{30}H_{48}$ (408)
25.77	9.3	Betulin	$C_{30}H_{50}O_2$ (442)
28.66	1.4	Ursa-9(11),12-dien-3-one	$C_{30}H_{46}O$ (422)
28.96	13.1	Hop-22(29)-en-3.beta.-ol	$C_{30}H_{50}O$ (426)
29.57	21.1	4,4,6a,6b,8a,11,12,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,1	$C_{30}H_{48}O$ (424)
		Leaf	
12.93	10.0	Bicyclo[3.1.0]hexan-2-one, 1,5-*bis*(1,1-dimethylethyl)-3,3-dimethyl-	$C_{16}H_{28}O$ (236)
17.76	7.6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$ (270)
19.55	15.0	Octadecanoic acid, methyl ester	$C_{19}H_{34}O_2$ (294)
21.20	6.3	Methyl 18-methylnonadecanoate	$C_{21}H_{42}O_2$ (326)
24.61	2.1	Di-*n*-decylsulfone	$C_{18}H_{36}O_2$ (284)
28.97	5.5	Triacontanoic acid, methyl ester	$C_{31}H_{62}O_2$ (466)

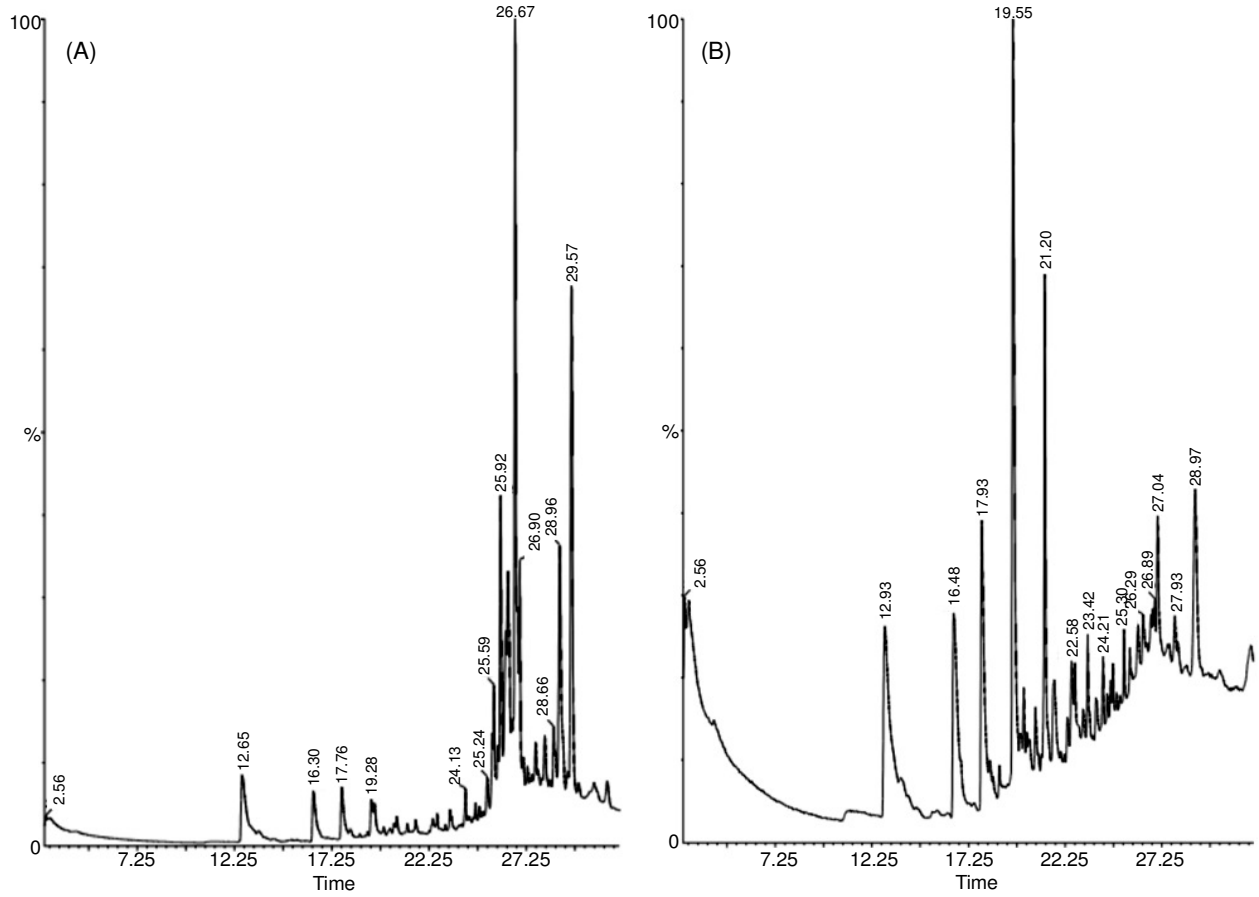

Fig. 3. A typical GC-MS chromatogram of bark (A) and leaf (B) fatty acids methyl ester

(1.2%), (z)-9-octadecanoic acid methyl ester (1.1 %), tetradecanoic acid, 12-methyl-, methyl ester, (s)- (0.9 %) in bark and octadecanoic acid, methyl ester (15.0 %), bicyclo[3.1.0]hexan-2-one, 1,5-*bis*(1,1-dimethyl-ethyl)-3,3-dimethyl-(10.0 %), hexadecanoic acid, methyl ester (7.6 %), methyl 18-methylnonadecanoate (6.3 %), and di-*n*-decylsulfone (2.1 %) in leaf has been confirmed by GC-MS analysis using NIST library. Both bark (Fig. 3A) and leaf (Fig. 3B) of *B. ovalifoliolata* was found to be rich in fatty acids.

Among the identified fatty acids methyl esters (17 compounds) (Table-2) tetradecanoic acid, *n*-hexadecanoic acid has antioxidant, anticancer, 5-α-reductase inhibitor, antimicrobial, hemolytic and antifibrinolytic activities.

Conclusion

In this study, the bioactive compounds present in *B. ovalifoliolata* was identified by GC-MS analysis which shows the presence of 17 fatty acids methyl ester in bark and leaf, similarly, GC-MS analysis of different crude extracts revealed the presence of 31 compounds.

REFERENCES

1. D. Gomathi, M. Kalaiselvi, G. Ravikumar, K. Devaki and C. Uma, *J. Food Sci. Technol.*, **52**, 1212 (2015); https://doi.org/10.1007/s13197-013-1105-9.
2. B.B. Mishra and V.K. Tiwari, *Eur. J. Med. Chem.*, **46**, 4769 (2011); https://doi.org/10.1016/j.ejmech.2011.07.057.
3. J. Balunas and A.D. Kinghorn, *Life Sci.*, **78**, 431 (2005); https://doi.org/10.1016/j.lfs.2005.09.012.
4. G.S. Citoglu and O.B. Acikara, in ed.: S. Dhanarasu, Column Chromatography for Terpenoids and Flavonoids, In: Chromatography and Its Applications, InTech, pp. 13-50 (2012).
5. D.H. Geetha, M. Rajeswari and I. Jayashree, *Asian Pac. J. Trop. Biomed.*, **3**, 985 (2013); https://doi.org/10.1016/S2221-1691(13)60190-2.
6. I.V. Rodrigues, J.N. de P. Souza, A.C.G. Silva, L.A. Chibli, V.A.R. Cabral, S.A. Viera Filho, F.F. Perazzo, A.G. Guimarães and G.H.B. de Souza, *Pharmacogn. J.*, **5**, 6 (2013); https://doi.org/10.1016/j.phcgj.2012.08.001.
7. M.Z. Siddiqui, *Indian J. Pharm. Sci*., **73**, 255 (2011); https://doi.org/10.4103/0250-474X.93507.
8. R. Chib, M. Kumar, M. Rizvi, S. Sharma, A. Pandey, S. Bani, S.S. Andotra, S.C. Taneja and B.A. Shah, *RSC Adv.*, **4**, 8632 (2014); https://doi.org/10.1039/c3ra46412a.
9. S.L. Deore and S.S. Khadabadi, *Phcog. Res.*, **2**, 343 (2010); https://doi.org/10.4103/0974-8490.75452.
10. L.H. Vazquez, J. Palazon, A.N. Ocana, in ed.: V. Rao, The Pentacyclic Triterpenes α,β-Amyrins: A Review of Sources and Biological Activities, In: Phytochemicals-A Global Perspective of Their Role in Nutrition and Health, InTech, Chap. 23. pp. 487-502 (2012).
11. S. Panda, M. Jafri, A. Kar and B.K. Meheta, *Fitoterapia*, **80**, 123 (2009); https://doi.org/10.1016/j.fitote.2008.12.002.
12. W. Maya, K. Mayur and S. Ashar, *Int. J. Pharm. Chem. Sci.*, **1**, 674 (2012).