



IDENTIFICATION OF COMPOUNDS FROM DIFFERENT FRACTIONS OF *EXACUM BICOLOR* ROXB. BY GC-MS ANALYSIS

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Abstract

Exacum bicolor Roxb. is a perennial herb belonging to the family Gentianaceae. The whole plant of *Exacum bicolor* Roxb. was collected, shade dried and coarsely powdered. The entire plant powder has been extracted by maceration with petroleum ether, chloroform, ethyl acetate and methanol. Each extract subjected to GC MS analysis and the petroleum ether extract chromatogram have shown the presence of 17 compounds; the chloroform extract showed 15 compounds, ethyl acetate containing 12 compounds and the methanolic extract consists of 10 compounds. The structure and uses of some bioactive constituents also discussed.

Key words: *Exacum bicolor* Roxb., GC-MS, petroleum ether, chloroform, ethyl acetate, methanol.

Introduction

The world's human population depends on herbal drugs from the ancient days onwards. The people use herbal medicines based on the belief that natural herbs are fewer side effects, less toxicity and more patient acceptance. The sources of natural medicines are plant sources, animal sources, marine sources and mineral sources. The most widely using source is plant sources and it consists of primary and secondary metabolites, which are responsible for the particular activity of the plant. The primary and secondary metabolites present in plants like carbohydrates, lipids, alkaloids, glycosides, tannins, resins, volatile oil, etc. Each plant contains more than one chemical constituents; according to this, they exhibit different pharmacological activity. For the identification of the secondary metabolite, the most commonly used method is the chromatographic technique.

The hyphenation of gas chromatography with mass spectroscopy is one of the methods for the identification of the active constituents of plant extracts. In GC MS analysis, the sample converted into vapor form and the capillary column packed with a stationary phase separates various components. Inert gases like argon, helium, or

nitrogen used as propellant gas and based on the retention time of the compound, they separate. After this, the components are entering the mass spectrometer and ionize the sample and the ionized samples accelerated through the instrument's mass analyzer. The ions are separated based on the mass to charge ratio; finally, the ions detected, analyzed and the final mass spectrum will obtain from the readout device.

Exacum bicolor Roxb. is an angiosperm belonging to the family Gentianaceae, which is a family of flowering plants with 84 genera and 1688 species. *Exacum bicolor* Roxb. is an erect herb growing from July to November in plains and July to January in high ranges. The flowering period of this plant in plain from September to November, whereas in high altitude, flowering will bear up to January. The unique feature of this plant is limited to a small area only even in a vast stretch of grasslands and the frequency of distribution is more in the dry pastures of plain compared to high altitude grasslands. In the plain dry grassland, it grows up to a height of 25-80cm. In high altitude grassland, it grows up to 40-120cm. The whole plant is having benefits such as, as a tonic, febrifuge and stomachic. The plant also yields a dye. Being bitter in taste, the local people take it as an herbal remedy for diabetes and skin disorder. In Kerala, the traditional practitioners prescribe

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the decoction of the whole plant for washing the eyes. However, due to the limited distribution and lack of availability of the plant throughout the year, it is not commonly used (Sreelatha, U. *et al.*, 2007).

Materials and Methods

Plant collection and authentication

The whole plant of *Exacum bicolor* Roxb. (Family: *Gentianaceae*) were collected in the month of August-November from Kannur district, Kerala, India. The plant was taxonomically identified and authenticated. A voucher specimen (17PH001R) placed for future reference in the

laboratory of Department of Pharmacognosy, Nitte Gulabi Shetty Memorial Institute of Pharmaceutical Sciences, Karnataka, India. The whole fresh plant was collected, washed and shade dried for two weeks at a temperature not exceeding 60°C to prevent the deactivation of thermolabile phytoconstituents.

Preparation of extracts

The dried plant was coarsely powdered, sieve through 60 mesh and stored in an airtight container at room temperature until further use. The powdered *Exacum bicolor* (200 gm) was extracted successfully by using petroleum ether, chloroform, ethyl acetate and methanol

Table 1: Chemical composition of Petroleum ether extract of *Exacum bicolor* Roxb.

Sl. No:	IUPAC Name of the compound	Retention time	Area %	Molecular formula	Molecular weight
1.	2-Pentadecanone, 6,10,14-trimethyl	17.254	5.634	C ₁₈ H ₃₆ O	268
2.	Sulfurous acid, dodecyl 2-propyl ester	21.176	1.497	C ₁₅ H ₃₂ O ₃ S	292
3.	4,8,12,16-Tetramethylheptadecan-4-olide	21.381	2.586	C ₂₁ H ₄₀ O ₂	324
4.	Tritetracontane	21.846	4.356	C ₄₃ H ₈₈	604
5.	Di-n-octyl phthalate	22.491	2.389	C ₂₄ H ₃₈ O ₄	390
6.	Nonadecane	22.551	5.044	C ₁₉ H ₄₀	268
7.	Heptacosane	23.227	10.192	C ₂₇ H ₅₆	380
8.	1,1'-Bicyclopentyl, 2-hexadecyl	24.392	2.905	C ₂₆ H ₅₀	362
9.	Tetratetracontane	24.527	14.008	C ₄₄ H ₉₀	618
10.	Hexatriacontane	25.162	7.651	C ₃₆ H ₇₄	506
11.	Tetracontane	25.773	10.581	C ₄₄ H ₉₀	618
12.	Pentatriacontane	26.413	5.446	C ₃₅ H ₇₂	492
13.	Sulfurous acid, 2-propyl tetradecyl ester	28.043	3.147	C ₁₈ H ₃₈ O ₃ S	334
14.	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-c	28.279	3.062	C ₃₀ H ₃₃ O ₆ Cl	524
15.	Nonadecane, 1-chloro	29.099	1.732	C ₁₉ H ₃₉ Cl	302
16.	2-Dodecen-1-yl(-)succinic anhydride	29.164	2.432	C ₁₆ H ₂₆ O ₃	266
17.	9,19-Cyclolanost-24-en-3-ol, acetate, (3.beta.)-	30.039	1.555	C ₃₂ H ₅₂ O ₂	468

Table 2: Chemical composition of Chloroform extract of *Exacum bicolor* Roxb.

Sl. No:	IUPAC Name of the compound	Retention time	Area %	Molecular formula	Molecular weight
1.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.454	8.482	C ₂₀ H ₄₀ O	296
2.	2,7-Octadiene-1,6-diol, 2,6-dimethyl-, (z)-	17.069	1.863	C ₁₀ H ₁₈ O ₂	170
3.	z,z-6,13-Octadecadien-1-ol acetate	17.784	3.822	C ₂₀ H ₃₆ O ₂	308
4.	1,2-Dipropylcyclopropene-3-carboxylic acid	18.100	21.313	C ₁₀ H ₁₆ O ₂	168
5.	Hexadecanoic acid, 2-oxo-, methyl ester	18.760	2.115	C ₁₇ H ₃₂ O ₃	284
6.	Hydroxylamine, o-decyl	20.320	7.203	C ₁₀ H ₂₃ ON	173
7.	1-Iodo-2-methylundecane	21.860	6.967	C ₁₂ H ₂₅ I	296
8.	Hexatriacontane	23.242	8.682	C ₃₆ H ₇₄	506
9.	Tritetracontane	23.922	4.184	C ₄₃ H ₈₈	604
10.	Squalene	24.082	2.082	C ₃₀ H ₅₀	410
11.	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate	24.422	1.690	C ₃₀ H ₃₃ O ₆ Cl	524
12.	Dotriacontane	24.557	8.522	C ₃₂ H ₆₆	450
13.	Heptacosane	25.803	5.497	C ₂₇ H ₅₆	380
14.	Heptacosane, 1-chloro-	27.198	2.798	C ₂₇ H ₅₅ Cl	414
15.	Octadecane, 1-chloro	28.084	1.870	C ₁₈ H ₃₇ Cl	288

by maceration. All the extracts were concentrated using a vacuum rotary evaporator and dried in a vacuum desiccator.

GCMS analysis

The GCMS analysis of Petroleum ether, chloroform,

ethyl acetate and methanol extract of *Exacum bicolor* performed. The investigation was carried out on a Perkin-Elmer workstation, with model Clarus 600 GC coupled to a mass spectrometer (Perkin Elmer Technologies, Inc., Wilmington, DE). Elite-5MS (30m × 0.25 mm width film

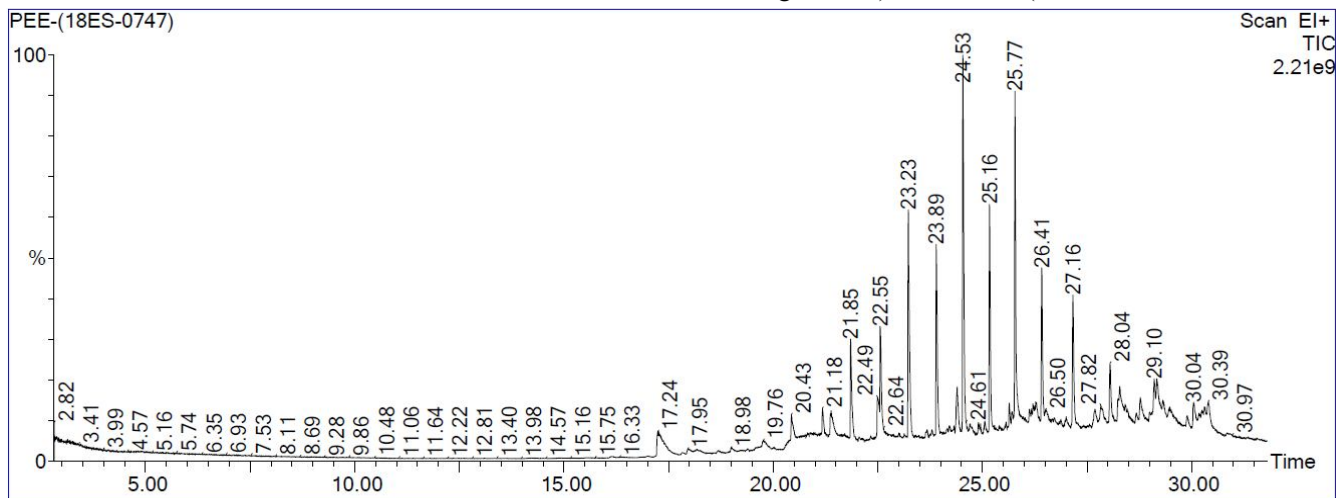


Fig. 1: GC-MS chromatogram of Petroleum ether extract of *Exacum bicolor* Roxb.

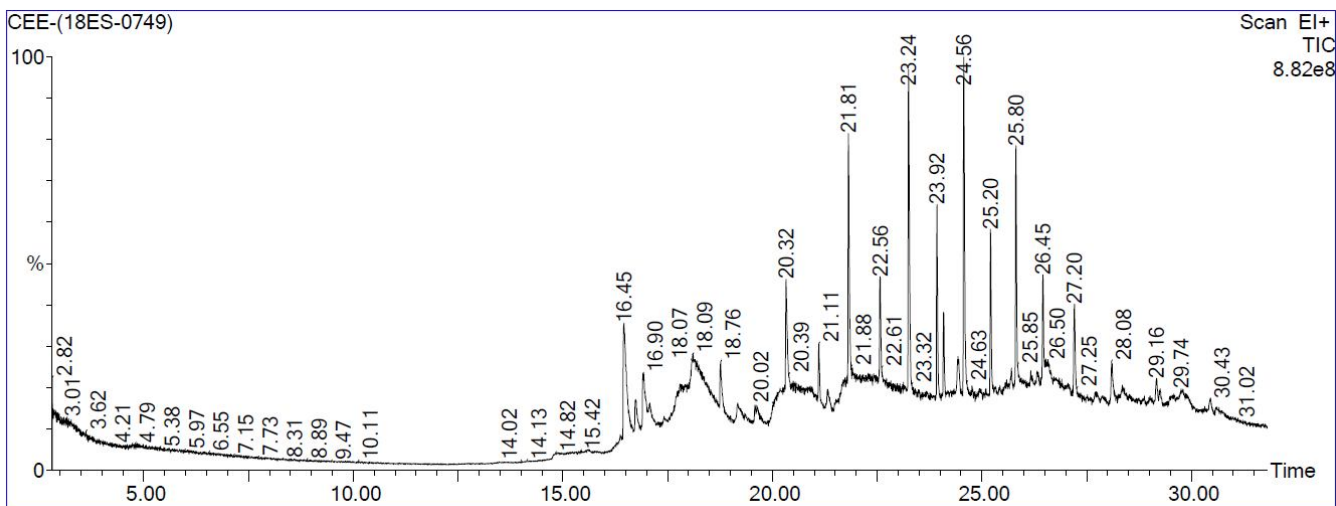


Fig. 2: GC-MS chromatogram of Chloroform extract of *Exacum bicolor* Roxb.

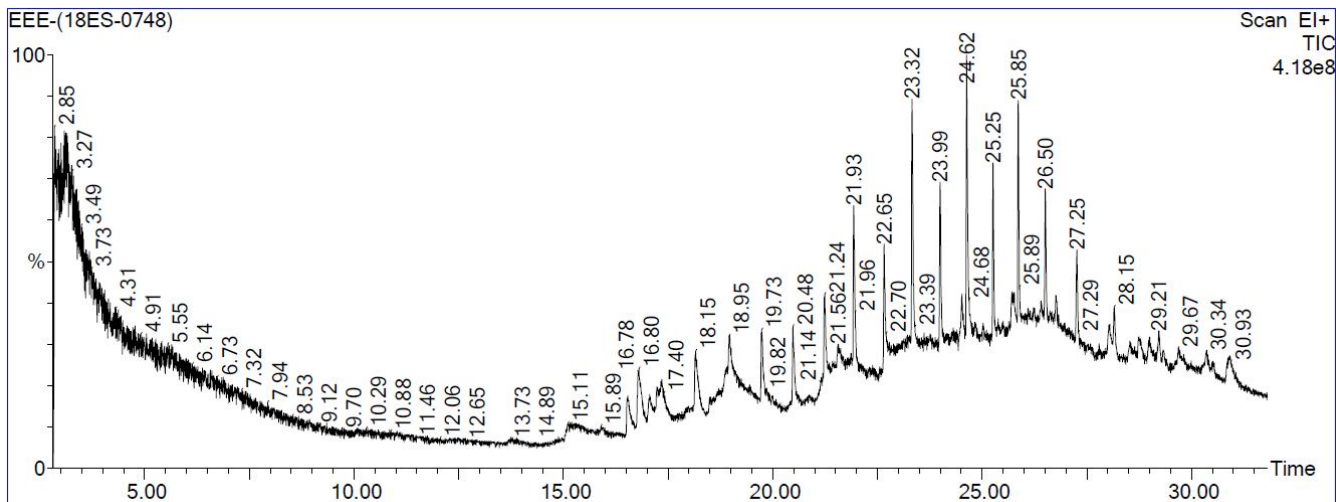


Fig. 3: GC-MS chromatogram of Ethyl acetate extract of *Exacum bicolor* Roxb.

Table 3: Chemical composition of Ethyl acetate extract of *Exacum bicolor* Roxb.

Sl. No:	IUPAC Name of the compound	Retention time	Area %	Molecular formula	Molecular weight
1	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	16.799	5.329	C ₂₀ H ₄₀ O	296
2	Behenic alcohol	18.155	5.801	C ₂₂ H ₄₆ O	326
3	Cis-.beta.-terpineol	18.965	12.532	C ₁₀ H ₁₈ O	154
4	Dotriacontane	20.476	4.275	C ₃₂ H ₆₆	450
5	17-Pentatriacontene	21.236	5.161	C ₃₅ H ₇₀	490
6	Sulfurous acid, 2-propyl tetradecyl ester	21.926	5.582	C ₁₇ H ₃₆ O ₃ S	320
7	Hexatriacontane	22.651	7.684	C ₃₆ H ₇₄	506
8	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	23.317	17.342	C ₂₆ H ₅₄	366
9	2,2-Dibromocholestanone	24.512	3.013	C ₂₇ H ₄₄ OBr ₂	542
10	Sulfurous acid, pentadecyl 2-propyl ester	24.622	9.256	C ₁₈ H ₃₈ O ₃ S	334
11	Heptacosane, 1-chloro	25.853	5.738	C ₂₇ H ₅₅ Cl	414
12	17-(1,5-Dimethylhexyl)-10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclopenta[a]phena	30.930	3.128	C ₂₇ H ₄₂ O	382

depth of 250µm capillary tube was used under the following condition.

Acquisition parameters

Oven : Initial temp 60°C for 2.80 min, ramp 10°C/min to 290°C, hold 6 min

InjAauto : 260°C

Split : 10:1

Carrier Gas : He

Solvent Delay : 2.80 min

Transfer Temp : 230°C

Source Temp : 230°C

Scan : 40 to 600Da

Column : 30.0m × 250µm

The interpretation of the GC-MS spectrum was carried out by comparing the spectrum of the unknown compound by the spectrum of known compounds in the

NIST library. The NIST - LIB 0.5 is an inbuilt software of GC-MS and having more than 62,000 patterns. Each compound was compared with the percentage score of the reverse and forward spectrum. The MS spectrum displays the molecular weight of individual molecules accurately (Ruthiran Papitha *et al.*, 2017).

Results and Discussion

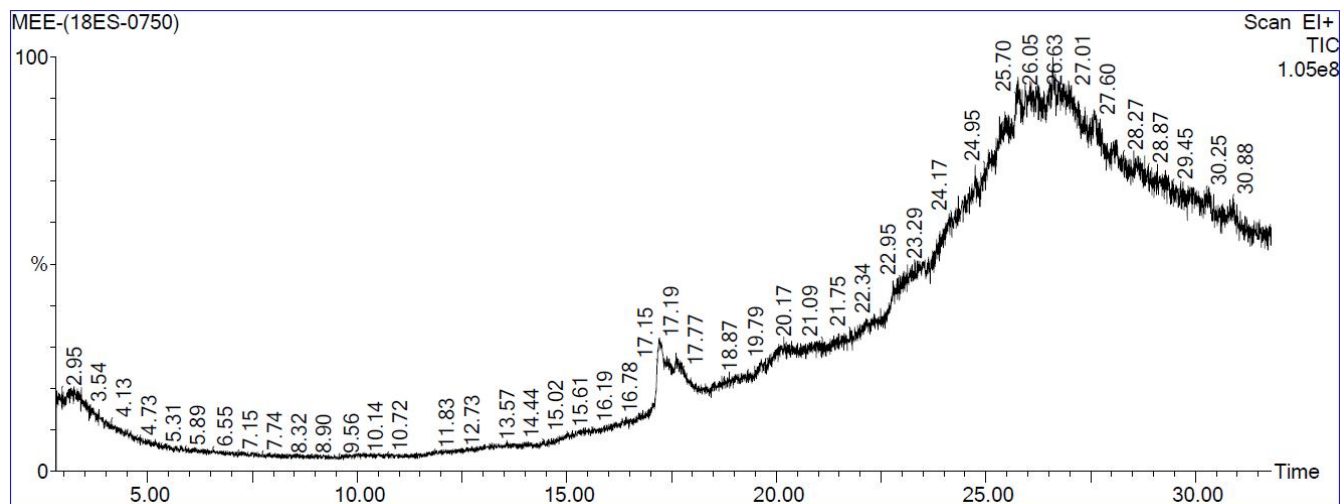
The GCMS analysis of petroleum ether extract, chloroform extract, ethyl acetate extract and methanol extract was carried out. The chromatogram of petroleum ether extract, chloroform extract, ethyl acetate extract and the methanol extracts are given in fig. 1, 2, 3 and 4, respectively. The petroleum ether extract has shown the presence of 17 compounds, chloroform extract has 15 compounds, ethyl acetate extract containing 12 compounds and the methanolic extract has 10 compounds, the IUPAC name, retention time, peak area %, molecular formula and molecular weight of the compound are

Table 4: Chemical composition of Methanol extract of *Exacum bicolor* Roxb.

Sl. No:	IUPAC Name of the compound	Retention time	Area %	Molecular formula	Molecular weight
1	Hexadecanal	17.204	2.736	C ₁₆ H ₃₂ O	240
2	7-Hydroxy-3-(1,1-dimethylprop-2-enyl)coumarin	24.737	2.073	C ₁₄ H ₁₄ O ₃	230
3	Cholest-8-en-3-ol, 14-methyl-, (3.beta.,5.alpha.)-	24.758	26.413	C ₂₈ H ₄₈ O	400
4	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3.beta.,17.beta.)-	25.758	3.371	C ₂₂ H ₃₂ O ₂	328
5	Pseudosarsasapogenin-5,20-dien	25.928	13.263	C ₂₇ H ₄₂ O ₃	414
6	Beta Carotene	26.048	4.003	C ₄₀ H ₅₆	536
7	Pregnan-3,11-diol-20-one	26.373	9.643	C ₂₁ H ₃₄ O ₃	334
8	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl carbonate	26.963	16.499	C ₃₀ H ₃₃ O ₆ Cl	524
9	Ethyl iso-allocholate	27.568	8.093	C ₂₆ H ₄₄ O ₅	436
10	Urs-12-en-28-ol	28.094	3.928	C ₃₀ H ₅₀ O	426

Table 5: Bioactive constituents with structure and uses.

S. No.	IUPAC name of the compound	Structure	Uses
1.	Nonadecane		Antioxidant
2.	Heptacosane		Anti-inflammatory activity
3.	Tetracontane		Anti-inflammatory activity
4.	Tetratetracontane		Antibacterial
5.	1-iodo-2-methylundecane		Antimicrobial activity
6.	Squalene		Antioxidant, cholesterol control, emollient, detoxifying and anticancer activity.
7.	Dotriacontane		Antimicrobial, antioxidant, antispasmodial
8.	17- Pentriacontane		Antibacterial and antiviral
9.	7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin		Anti-quorum sensing activity
10.	Beta carotene		Precursor for vitamin A synthesis.
11.	Ethyl Iso-Allocholate		Antimicrobial, diuretic, anti-inflammatory, antiasthmatic


Fig. 4: GC-MS chromatogram of Methanol extract *Exacum bicolor* Roxb.

mentioned in table 1, 2, 3 and 4 respectively. Tritetracontane, heptacosane were present in both petroleum ether and chloroform extract. Heptacosane, 1 chloro- is present in chloroform and ethyl acetate extract. Hexatriacontane is present in petroleum ether, chloroform and ethyl acetate extract.

The petroleum ether extract contain about 17 compounds in which tetracontane having maximum peak percentage area of 14.008, tetracontane 10.581, Heptacosane 10.192, Hexatriacontane 7.651, 2-Pentadecanone, 6,10,14-trimethyl 5.634, Pentatriacontane 5.446 are the other major constituents. The main active principles present in chloroform extract are 1,2-dipropylcyclopropene-3-carboxylic acid with a peak area percentage of 21.313, followed by Hexatriacontane (8.682), Dotriacontane (8.522), 3,7,11,15-tetramethyl-2-hexadecen-1-ol (8.482), 1-iodo-2-methylundecane (6.967), Heptacosane (5.497), Squalene (2.082). Octadecane, 3-ethyl-5-(2-ethylbutyl) 17.342, Cis-beta-terpineol 12.532, sulfurous acid, pentadecyl 2-propyl ester 9.256, Hexatriacontane 7.684, Behenic alcohol 5.801, sulfurous acid, 2-propyl tetradecyl ester 5.582 are the major constituents of ethyl acetate extract. The methanolic extract consists of polar constituents like cholest-8-en-3-ol, 14-methyl-, (3.beta.,5.alpha.) 26.413, 2-isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-coumarin-4-yl 00carbonate 16.499, psuedosarsasapogenin-5,20-dien 13.263, pregnan-3,11-diol-20-one 9.643, ethyl iso-allocholate 8.093, Beta Carotene 4.003, Urs-12-en-28-ol 3.928, Hexadecanal 2.736, etc.

The tetracontane has anti-inflammatory activity (Sunita Arora and Ganesh Kumar, 2017). Heptacosane volatile aliphatic hydrocarbon with antibacterial activity, 1-iodo-2-methylundecane is an organoiodide with antimicrobial activity and squalene possesses antioxidant, cholesterol control, emollient, detoxifying and anticancer activity (Gopinath, S. *et al.*, 2013). Triacontane possesses antibacterial, antidiabetic and antitumor activities. The octadecane, 3-ethyl-5-(2-ethyl butyl) is used as an antifungal and antimicrobial agent (Arockia Jenecius A. *et al.*, 2012). Dotriacontane is an antimicrobial, antioxidant, antispasmodic agent; behenic alcohol possesses antiviral activity, pentatriacontane having antibacterial and antiviral activity, hexadecane with multiple uses like antimicrobial, antioxidant and antidiabetic activity (Paramanatham, M. and Murugesan, A., 2014). 7-Hydroxy-3-(1,1-dimethylprop-2-enyl) coumarin recently investigated for anti-quorum sensing activity (Rajesh p Shastry and Mohammed Aman, 2019). Beta

carotene is a starting material for vitamin A synthesis. Ethyl iso-allocholate has Antimicrobial, diuretic, anti-inflammatory, antiasthmatic activity (Muthulaxmi, A. *et al.*, 2012).

Conclusion

The GC-MS analysis of petroleum ether, chloroform, ethyl acetate and methanol extracts of *Exacum bicolor* Roxb. revealed the presence of different active principles with different pharmacological action. So, this whole-plant extract can be used for treating multiple diseases. However, further studies will require the isolation and characterization of individual phytoconstituents and investigating its pharmacological activity and mechanism of action.

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