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CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN THE METHANOLIC EXTRACT OF MOSS *HERPETINEURON TOCCOAE* (SULL. & LESQ.) CARDOT USING GC-MS ANALYSIS

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ABSTRACT

Bryophytes are the simplest and most primitive land plants due to absence of conductive tissue system. Being small, this group of plants have been neglected for a long time. The pharmaceutical investigations of their chemical compositions in last few decades have proven their medicinal importance. *Herpetineuron toccoe* (Sull. & Lesq.) Cardot is a plerocarpic moss belonging to family Anomodontaceae. The moss is cosmopolitan in distribution. The objective of present study is to analyze the presence of bioactive secondary metabolites from the methanolic extract of *Herpetineuron toccoe*. The phytochemical screening was carried out through gas chromatography- mass spectrometry technique. A total of 28 bioactive phytochemical compounds were isolated from the methanolic extract. These compounds were Pterin-6-carboxylic acid, Thieno [2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-, Ethylbenzene, Dodecylcyclohexane, 1,7-Dimethyl-4-(1-methylethyl)cyclodecane, 1- Hexadecanol, 2-methyl, cis-13-Eicosenoic acid, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, trans-13- Octadecenoic acid, 3-Trifluoroacetoxypentadecane, n-Tetracosanol-1, 2-Hexyl-1-octanol, 1-Hexadecanol, 8-Octadecenal, 5-Octadecenal, 9-Hexadecenoic acid, 1-Hexadecanol, 2-methyl-, 17-Pentatriacontene, 10-Octadecenal, Cholestan-3-one, cyclic 1,2-ethanediy aetal, (5a_i)-, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, Ethyl iso-allocholate, cis-11-Eicosenoic acid, 1-Heptatriacotanol, Cyclopropanedodecanoic acid, 2-octyl-, methyl ester, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3a_i,5Z,7E)-, Oleic acid, 3-(octadecyloxy)propyl ester. The identification of these bioactive phytochemical compounds was based on peak area, retention time, molecular formula and weight. Subsequently, anti-microbial, anti-inflammatory, anti-cancerous properties of the extracted phytochemicals indicate the medicinal value of *Herpetineuron toccoe*.

Key words : Bryophytes; *Herpetineuron toccoe*, Bioactive compounds, GC-MS analysis, Phytochemicals.

Introduction

The significance of natural and herbal medicines is increasing day by day as people are now more attracted towards them due to their potential, cost effectiveness and least side effects. Almost 80% population worldwide depends on herbal medication directly or indirectly (Ekor, 2014). Plants are rich source of active ingredients called secondary metabolites having anti-microbial, anti-inflammatory, anti-cancerous activities, which makes them pharmacologically important (Chavhan, 2017). The screening of plants is important to detect the presence of

bioactive compounds, which can be used in pharmacological studies. However, the method used for identification should be easy and repeatable (Ganesh *et al.*, 2017). Gas chromatography- mass spectrometry technique can separate volatile compounds very efficiently, thus, making it very compatible for identification and quantification prospect (Vinodh *et al.*, 2013).

Herpetineuron toccoe (Sull. & Lesq.) Cardot is yellowish- green plant, growing in compact turfs. Stem is ±5cm long, horizontal. Rhizoids are present. Stem is covered with leaves. The secondary branches emerging

from the main stem are erect and branched. Leaves are compactly arranged, lanceolated with acuminate tip and broad base, margins serrated in the upper region. The presence of tortuous vein inspired Cardot to name this moss, *Herpetineuron* (snake costa). Costa is the main identifying feature of this genus. It is strong, ends almost at the apex. Laminal cells are thick, almost hexagonal in shape. Perichaetial leaves are present. Setae erect with slightly inclined capsule. Peristome teeth double. The moss is cosmopolitan in distribution.

Many plants especially bryophytes are phytochemically unexplored as compared to other plant groups. Due to the fact that bryophytes are small, simple plants and shortfall of the knowledge of their contribution towards the ecosystem, they have always been overlooked by conservation groups. Phytochemical studies on liverworts have shown the presence of various bioactive compounds which shows anti-bacterial, anti-fungal and anti-cancerous properties (Asakawa, 1995). However, a very little work has been done on the phytochemical analysis of mosses. The literature study revealed that no GC-MS analysis has been done so far on *Herpetineuron toccoae*. Therefore, the present work has been taken up to reveal the presence of phytochemicals, which are medicinally important.

Materials and Methods

Collection and preparation of plant material

The sample was collected from various locations of the study area (District Solan, Himachal Pradesh). The collected plant was identified and the specimen was deposited under PAN no. 6410 in the herbarium, Department of Botany, Panjab University, Chandigarh.

Preparation of plant extract

To prepare the methanolic extract of *Herpetineuron*

toccoae, 500mg of plant material was crushed in methanol and kept on rotatory shaker for 24 hrs. The filtrate was then subjected to GC-MS.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis of whole plant ethanol extract was done using Thermo Trace 1300GC with thermo TSQ8000 Triple Quadrupole MS at CIL/SAIF, P.U. Chandigarh with 250°C injector temperature, 10ul injector volume with 29.08 run time. TG 5MS (30m × 0.25 mm, 0.25um) column with 5% diphenyl and 95% dimethyl polysiloxane column make up was used for the analysis. In this chromatography, helium works as a carrier gas at 1.5ml/minute constant flow with 45ml/min split flow because in GC extract separated through heating and heated gases of individual substances are carried out to column through helium (an inert gas). Mass spectrum was taken at 70eV in 50-700 range along with MS transfer line temperature of 280°C. Ion source temperature was 230°C.

Identification of compounds

The obtained mass spectra were interpreted by comparing with National Institute of Standards and Technology (NIST) database. NIST 2.0 is a compendium of 62000 mass spectrum patterns which denotes chemical compounds (Kumar *et al.*, 2018). The bioactivity of these compounds was documented from the literature.

Results

The GC-MS analysis of *Herpetineuron toccoae* has shown the presence of 28 bioactive compounds. Results showed the identified compounds along with their molecular weight, retention time, area percentage and their biological activities and full GC-MS chromatogram. (Table 1, Fig. 1).

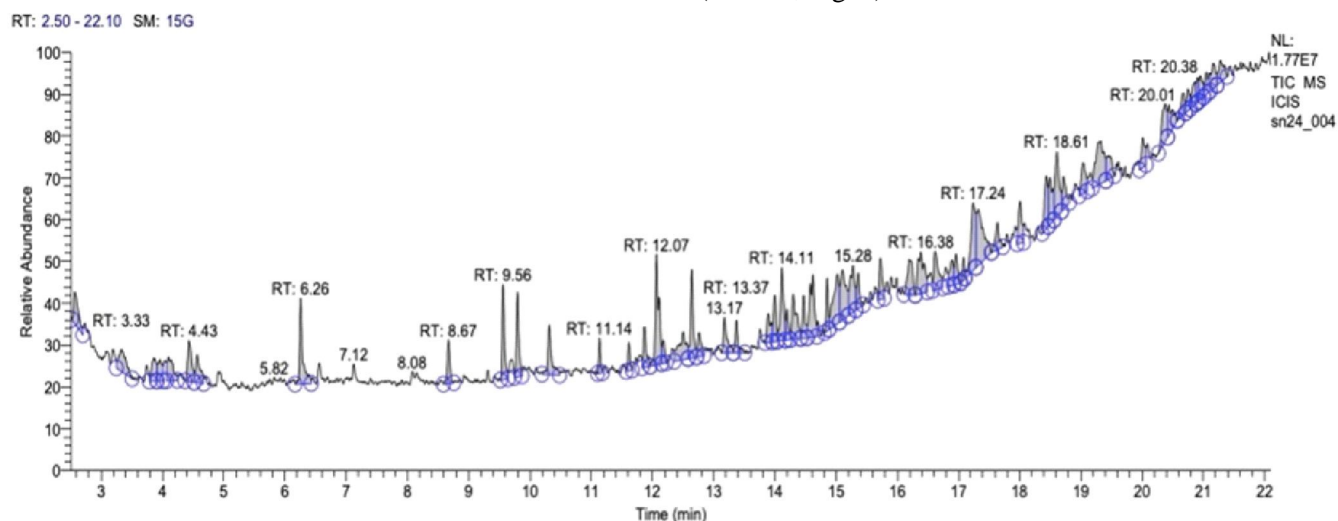


Fig. 1 : GC-MS chromatograph of methanolic extract of *H. toccoae*.

Table 1 : Bio-active compounds identified in *H. toccooe* by GC-MS analysis along with their biological activity.

S. no.	Compound name	Molecular Formula	RT	Area %	Biological activity	Reference
1.	Pterin-6-carboxylic acid	C7H5N5O3	3.33	1.63	Anti-cancerous	Kumar <i>et al.</i> (2018)
2.	Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-	C11H14N2OS	3.86	1.11	Analgesic, Antianginal, Analgesic, non-opioid, Antihypertensive, Antiarthritic, Dementia treatment, Neurotransmitter uptake inhibitor	Brintha <i>et al.</i> (2017)
3.	Ethylbenzene	C8H10	4.43	1.64	Used as solvents in insecticide sprays, rubber and chemical manufacturing, and household degreasers, paints, adhesives and rust preventives. As an antiknock agent in aviation and motor fuels	Jang <i>et al.</i> (2001)
4.	Dodecylcyclohexane	C18H36	10.31	1.86	As a source of carbon and energy for cultures	Beam and Perry (1974)
5.	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	C15H30	12.07	3.92	Anti-microbial, Anti-inflammatory	Krishnamoorthy and Subramaniam (2014)
6.	1-Hexadecanol, 2-methyl-	C17H36O	12.50	1.93	Anti-inflammatory	Kim <i>et al.</i> (2013)
7.	cis-13-Eicosenoic acid	C20H38O2	12.76	0.88	Oleochemical used in Pharmaceutical, cosmetic and food applications	Kassab <i>et al.</i> (2019)
8.	Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-	C9H9F3O2	13.89	1.11	Antioxidant, Anti-thrombotic and Anti-tuberculosis activity	Hameed <i>et al.</i> (2015)
9.	trans-13- Octadecenoic acid	C18H34O2	14.30	2.01	Anti-inflammatory, Anti- androgenic, Dermatitigenic, Anaemiagenic, Insecticides	Abubakar and Majinda (2016)
10.	3-Trifluoroacetoxypentadecane	C17H31F3O2	14.47	1.15	Anti-nephrotoxic, Anti-oxidant activities	Hussein <i>et al.</i> (2016)
11.	n-Tetracosanol-1	C24H50O	14.85	1.04	Antioxidant	Lakshmi and Nair (2017)
12.	2-Hexyl-1-octanol	C14H30O	15.02	2.83	Anti-microbial	Witkowska-Banaszczak and Dlugaszewska (2017)
13.	1-Hexadecanol	C16H34O	15.10	2.85	Used in surfactants, lubricants, detergents, pharmaceuticals and cosmetics.	Feng <i>et al.</i> (2016)
14.	8-Octadecenal	C18H34O	15.28	2.23	Anti-inflammatory activity, Anti-microbial activity	Hussein <i>et al.</i> (2016)
15.	5-Octadecenal	C18H34O	16.19	1.85	Food flavouring agent, Anti-microbial, Anti-inflammatory	Anjali <i>et al.</i> (2019)
16.	9-Hexadecenoic acid	C16H30O2	16.89	1.00	Non-cytotoxic	Ismail <i>et al.</i> (2013)
17.	1-Hexadecanol, 2-methyl-(terpenoid)	C17H36O	17.24	3.25	Anti-microbial	Sarada <i>et al.</i> (2011)

Table 1 continued...

Table 1 continued...

18.	17-Pentatriacontene	C35H70	17.33	3.83	Anti-inflammatory, Anti-cancerous, Anti-bacterial, Anti-arthritis	Kumar <i>et al.</i> (2018)
19.	10-Octadecenal	C18H34O	17.64	1.08	Anti-bacterial	Odiase-Omoighe and Agoreyo (2022)
20.	Cholestan-3-one, cyclic 1,2-ethanediyl acetal, (5a)	C29H50O2	18.44	1.78	Analgesic, Anti-ulcer, Anti-cancerous	Al-Rubaye <i>et al.</i> (2017)
21.	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	C26H54	18.50	1.73	Anti-oxidant and Anti-inflammatory effect	Al-Marzoqi <i>et al.</i> (2015)
22.	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	C18H28O3	18.61	2.71	Anti-fungal, Anti-oxidant activities	Bashir <i>et al.</i> (2015)
23.	Ethyl iso-allocholate	C26H44O5	18.72	1.16	Anti-microbial, Anti-inflammatory	Hameed <i>et al.</i> (2014)
24.	cis-11-Eicosenoic acid	C20H38O2	19.29	3.58	Acts as raw material for medical supplies and cosmetic creams.	Kikukawa <i>et al.</i> (2015)
25.	1-Heptatriacotanol	C37H76O	19.45	1.25	Anti-microbial	Kalaiarasan <i>et al.</i> (2017)
26.	Cyclopropanedodecanoic acid, 2-octyl-, methyl ester	C24H46O2	20.01	1.13	Anti-oxidants, Anti-microbial	Al-Rubaye <i>et al.</i> (2017)
27.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3a,5Z,7E)-	C27H44O3	20.38	1.97	Vitamin, Antipsoriatic, Bone diseases treatment, Antieczematic, Autoimmune disorders treatment	Brintha <i>et al.</i> (2017)
28.	Oleic acid, 3-(octadecyloxy)propyl ester	C39H76O3	20.44	1.35	Anti-microbial	Abubacker and Devi (2014)

The analysis revealed the presence of bioactive compounds, which includes Pterin-6-carboxylic acid, Thieno [2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-, Ethylbenzene, Dodecylcyclohexane, 1,7-Dimethyl-4-(1-methylethyl)cyclodecane, 1-Hexadecanol, 2-methyl, cis-13-Eicosenoic acid, Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, trans-13-Octadecenoic acid, 3-Trifluoroacetoxypentadecane, n-Tetracosanol-1, 2-Hexyl-1-octanol, 1-Hexadecanol, 8-Octadecenal, 5-Octadecenal, 9-Hexadecenoic acid, 1-Hexadecanol, 2-methyl-, 17-Pentatriacontene, 10-Octadecenal, Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5a)-, Octadecane, 3-ethyl-5-(2-ethylbutyl)-, Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, Ethyl iso-allocholate, cis-11-Eicosenoic acid, 1-Heptatriacotanol, Cyclopropanedodecanoic acid, 2-octyl-, methyl ester, 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3a,5Z,7E)-, Oleic acid, 3-(octadecyloxy)propyl ester. Moreover, the GC-MS spectrum of the identified phytochemicals are depicted individually in Fig. 2.

Discussion

The identified bioactive compounds possess various biological activities, which have significant use in pharmaceutical studies such as Pterin-6-carboxylic acid (Brintha *et al.*, 2017), 17-Pentatriacontene (Bertel *et al.*, 2021) and Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5a)- (Al-Marzoqi *et al.*, 2015) shows anti-cancerous activities. The compounds 1,7-Dimethyl-4-(1-methylethyl)cyclodecane (Kim *et al.*, 2013), 2-Hexyl-1-octanol (Feng *et al.*, 2015), 8-Octadecenal (Anjali *et al.*, 2019), 5-Octadecenal (Ismail *et al.*, 2013), 1-Hexadecanol, 2-methyl- (Odiase-Omoighe *et al.*, 2022), Ethyl iso-allocholate (Abubakar *et al.*, 2016), 1-Heptatriacotanol (Abubacker *et al.*, 2014), Cyclopropanedodecanoic acid, 2-octyl-, methyl ester (Al-Marzoqi *et al.*, 2015) and Oleic acid, 3-(octadecyloxy) propyl ester (Abubacker *et al.*, 2014) have been reported for their anti-microbial properties. The

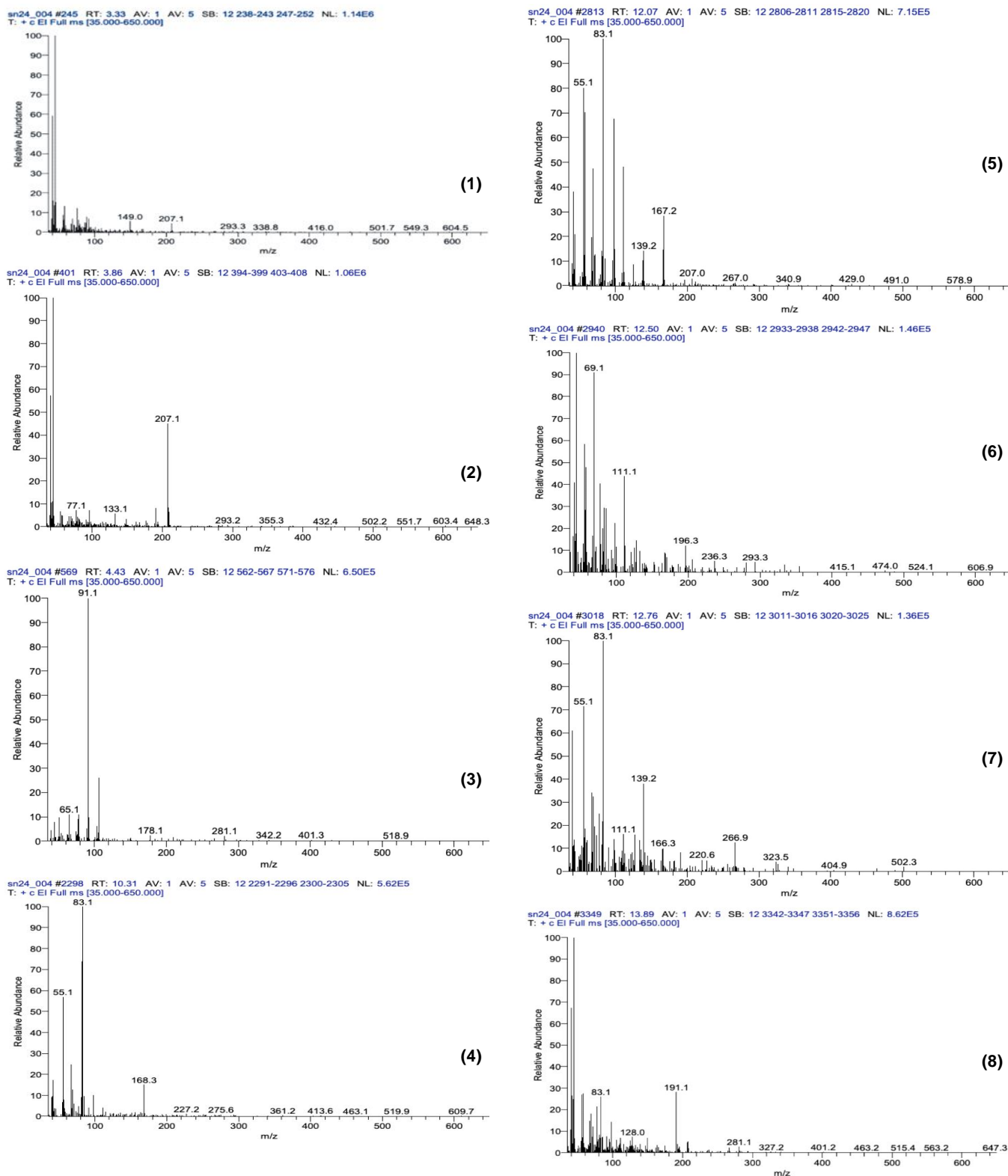


Fig. 2 : **1.** Pterin-6-carboxylic acid, **2.** Thieno[2,3-c]furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-tetramethyl-, **3.** Ethylbenzene, **4.** Dodecylcyclohexane, **5.** 1,7-Dimethyl-4-(1-methylethyl)cyclododecane, **6.** 1- Hexadecanol, 2-methyl, **7.** cis-13-Eicosenoic acid, **8.** Phen-1,4-diol, 2,3-dimethyl-5-trifluoromethyl-, **9.** trans-13- Octadecenoic acid, **10.** 3-Trifluoroacetoxypentadecane, **11.** n-Tetracosanol-1, **12.** 2-Hexyl-1-octanol, **13.** 1-Hexadecanol, **14.** 8-Octadecenal, **15.** 5-Octadecenal, **16.** 9-Hexadecenoic acid, **17.** 1-Hexadecanol, 2-methyl-, **18.** 17-Pentatriacontene, **19.** 10-Octadecenal, **20.** Cholestan-3-one, cyclic 1,2-ethanediyl aetal, (5*ai*)-, **21.** Octadecane, 3-ethyl-5-(2-ethylbutyl)-, **22.** Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester, **23.** Ethyl iso-allocholate, **24.** cis-11-Eicosenoic acid, **25.** 1-Heptatriacontanol, **26.** Cyclopropanedodecanoic acid, 2-octyl-, methyl ester, **27.** 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3*ai*,5*Z*,7*E*)-, **28.** Oleic acid, 3-(octadecyloxy)propyl ester. *Fig. 2 continued...*

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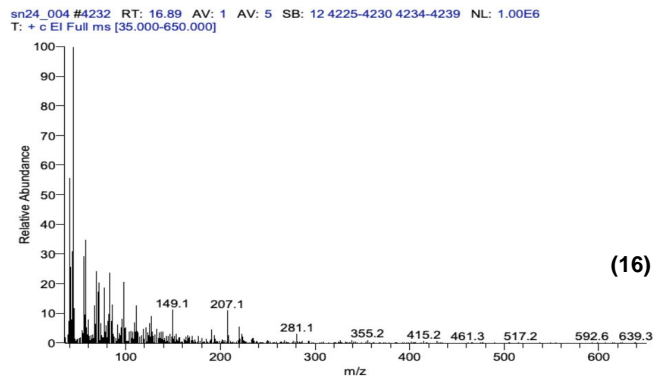
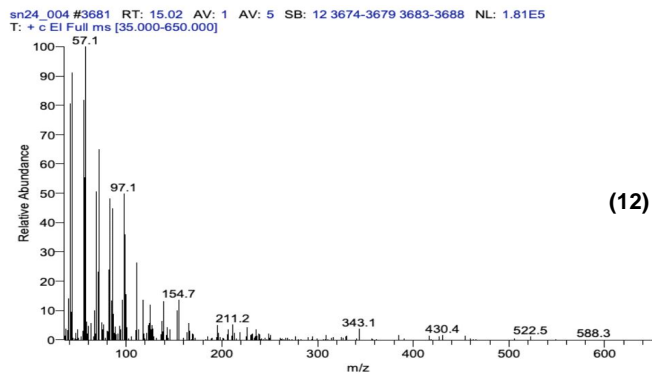
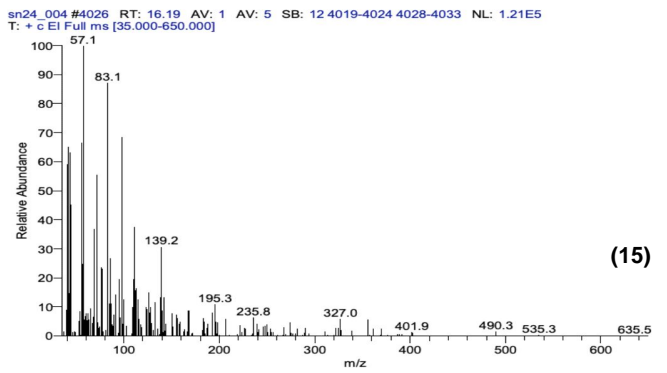
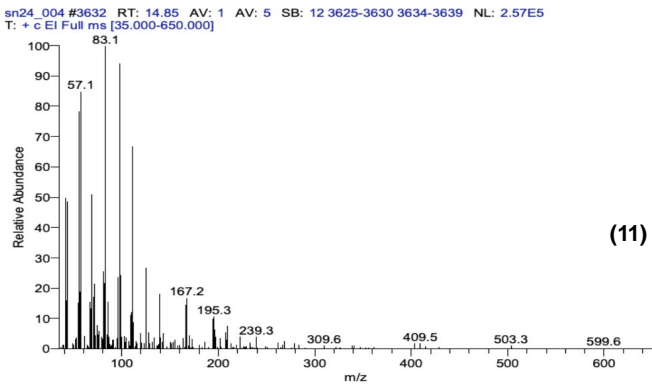
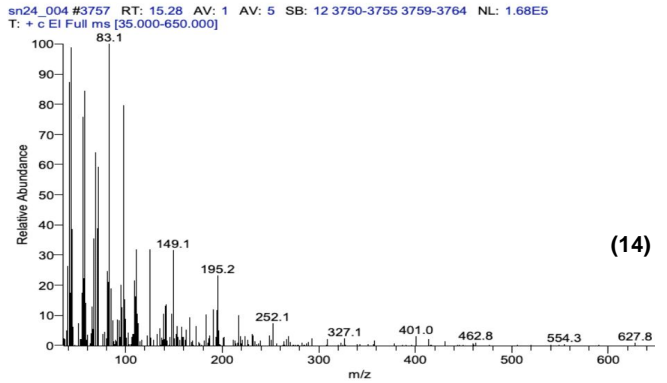
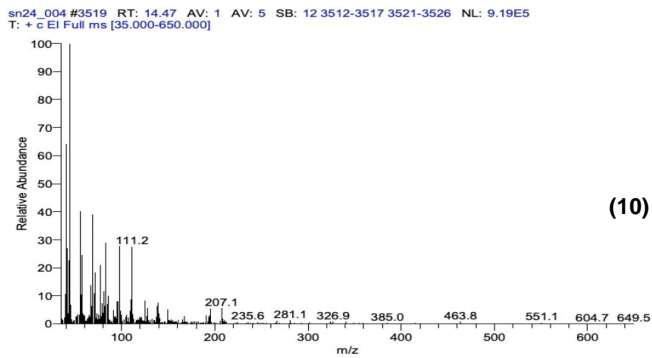
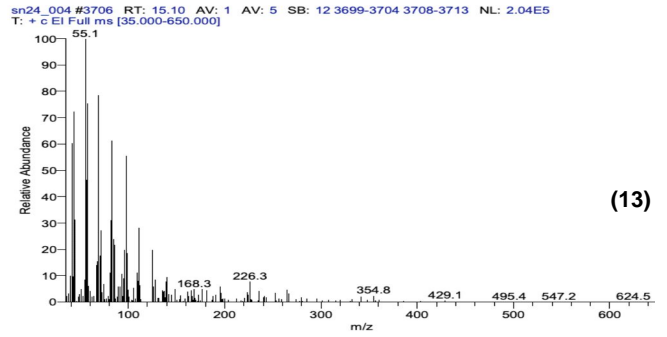
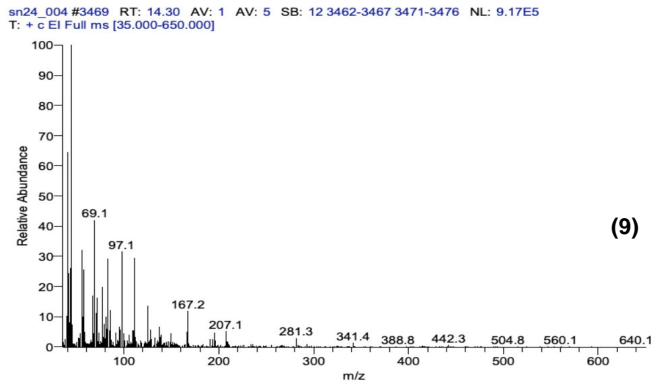


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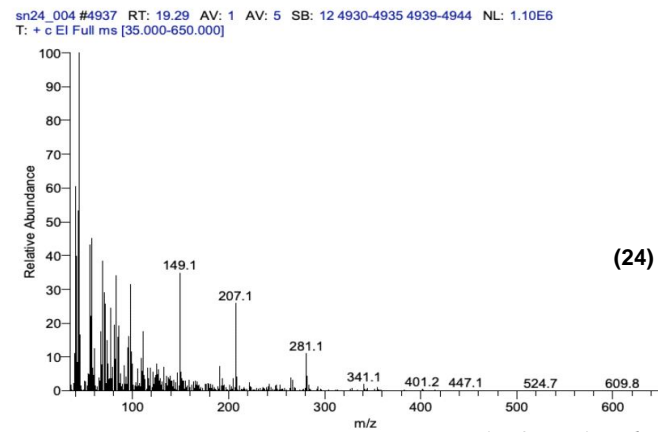
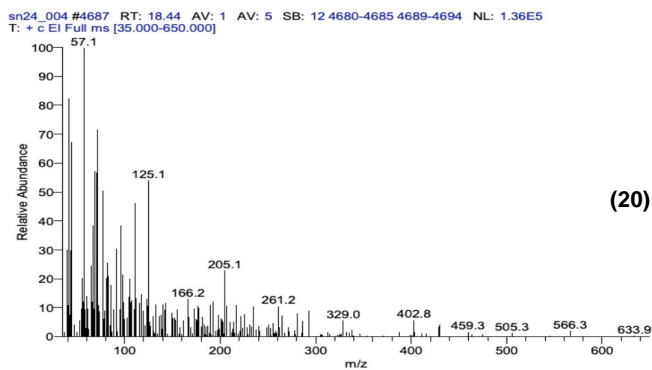
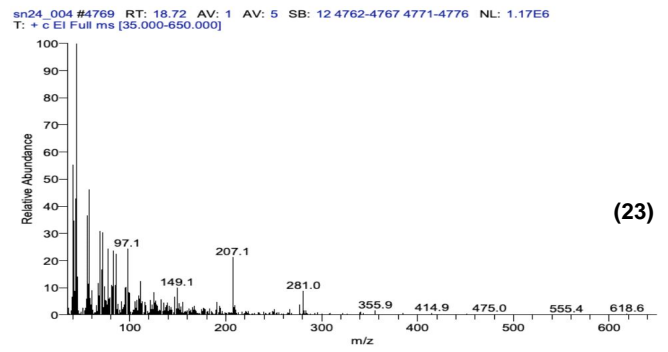
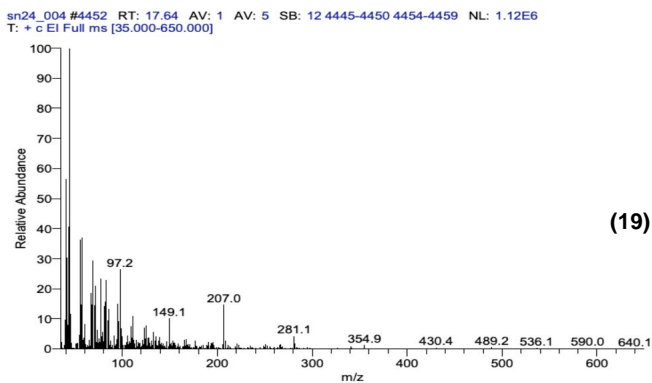
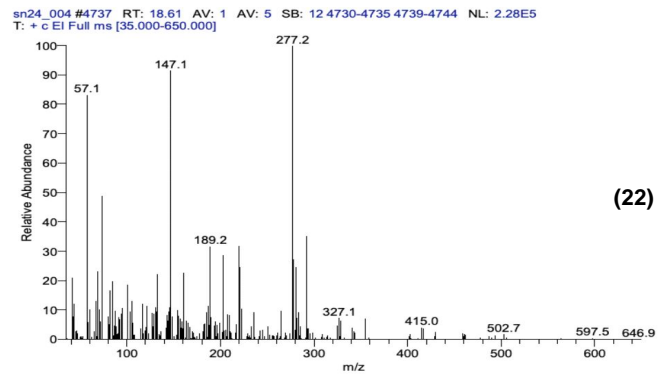
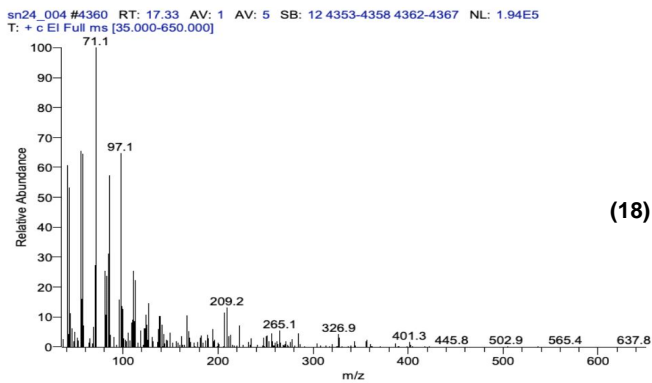
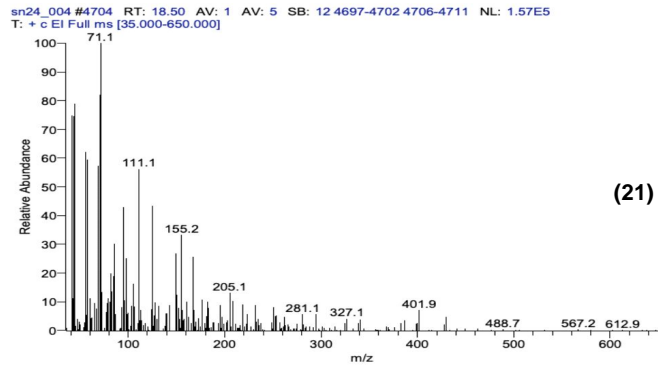
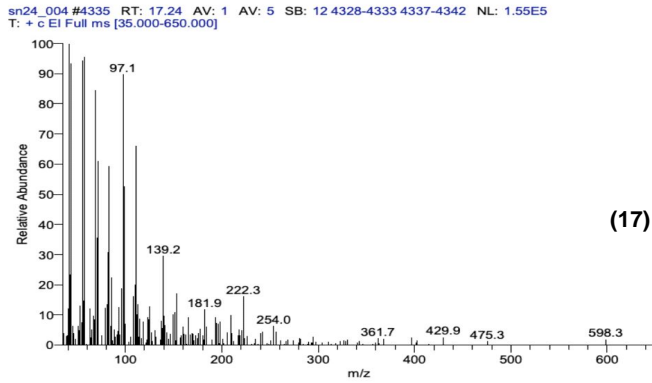
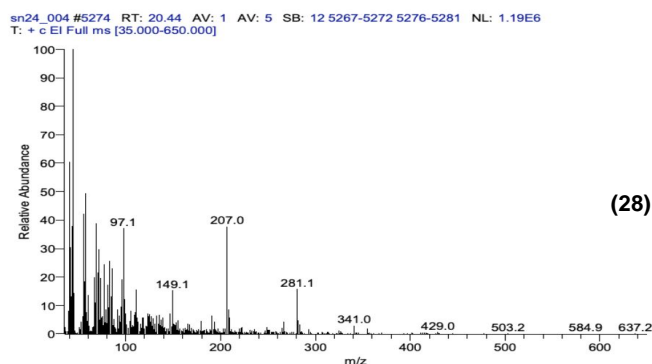
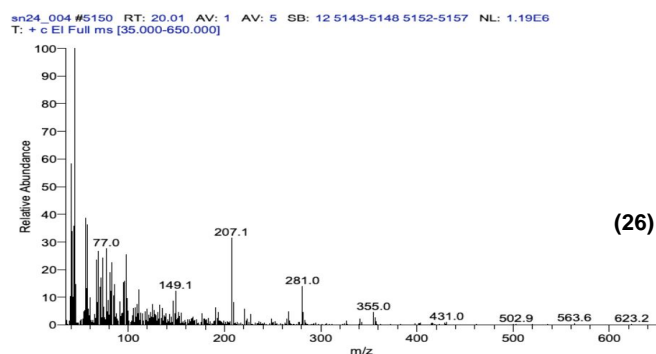
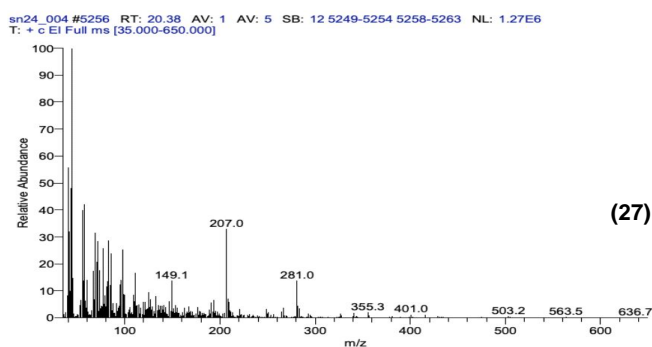
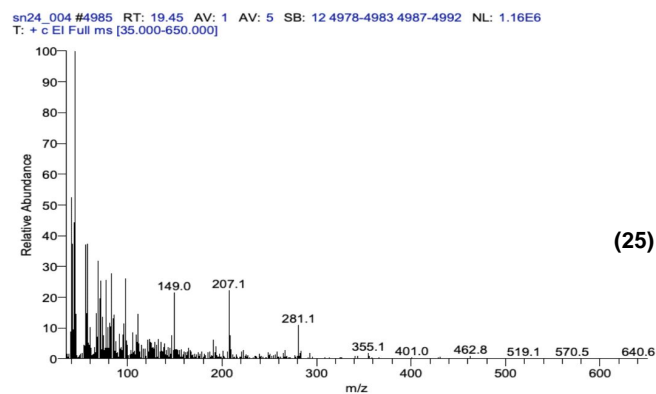


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compounds showing anti-inflammatory properties such as 1,7-Dimethyl-4-(1-methylethyl) cyclodecane (Kim *et al.*, 2013), 1-Hexadecanol, 2-methyl- (Kassab *et al.*, 2019), trans-13- Octadecenoic acid (Hussein *et al.*, 2016), 8-Octadecenal (Anjali *et al.*, 2019), 5-Octadecenal (Ismail *et al.*, 2013), 17-Pentatriacontene (Bertel *et al.*, 2021), Octadecane, 3-ethyl-5-(2-ethylbutyl)- (Bashir *et al.*, 2012) and Ethyl iso-allocholate (Abubakar *et al.*, 2016) were also found to be present in the plant extract. Moreover, the compound cis-11-Eicosenoic acid has been used as raw material for medical supplies and cosmetic industry (Kalaiarasan *et al.*, 2011). 1-Hexadecanol is used as surfactants, lubricants, detergents, pharmaceuticals and cosmetic industry (Hussein *et al.*, 2016).

Conclusion

A total of 28 biologically active compounds were isolated from methanolic extract of *Herpetineuron toccocae*. The results have clearly shown the importance of the studied moss in pharmaceutical, cosmetic and food industry.

Acknowledgments

Meenal Sharma sourced the plant materials, collected the data and wrote the paper. Shiwani Latwal contributed to data and helped in the analysis tools. Anju Rao conceived and designed the analysis. All the authors read and approved the manuscript.

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