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GC-MS ANALYSIS OF THE METHANOLIC EXTRACT OF *HYPOTRACHYNA INFIRMA* (KUROK) HALE

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ABSTRACT

Lichens are known to synthesize a variety of secondary metabolites having varied activity in response to external environmental conditions. The use of lichens in medicine is due to their secondary metabolites that are unique compared to those of higher plants. Hence, the present study was designed to determine the bioactive compounds in the methanol extract of less explored lichen species *Hypotrachyna infirma* that has been collected from non-polluted, high altitude area. Gas chromatography-mass spectrometry (GC-MS) analysis of methanol extract of *Hypotrachyna infirma* was investigated using Perkin-Elmer Gas chromatography-Mass spectrometry. The GC-MS analysis has shown the presence of different phytochemical compounds in methanol extract of *Hypotrachyna infirma* composition. A total of 40 compounds were identified with valuable biological activity in the above extract. From the results, it is evident that *Hypotrachyna infirma* contain various phytocomponents and is recommended as a lichen of phytopharmaceutical importance.

KEYWORDS

Hypotrachyna infirma, Gas Chromatography, Phytocomponents and Phytopharmaceutical Importance.

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INTRODUCTION

Lichens are symbiotic organisms consisting of a fungus partner and a photosynthetic organism, either an alga or Cyanobacteria[1,2]. They are ubiquitous on barks, stems, leaves and in soil but often grow in habitats that are less favorable for higher plants[3]. These organisms have historically been used as a cure for human diseases, food, dyes, in the production of alcohol and in the perfume industry[4]. Lichens are traditionally used in Chinese as well as in Indian Ayurveda for treating many ailments[5] as they possess some unique secondary

chemical compounds, which are lacking in higher plants.

Indian lichen biota is dominated by crustose lichens with (1518 taxa), followed by foliose (705 taxa) and fruticose (205 taxa) lichens. India has got a total of 35 states and Union Territories, of which Tamil Nadu records the maximum numbers of lichens with 785 taxa followed by Uttarakhand (581 spp.), West Bengal (531 spp.) and Sikkim (503 spp.). Location of lichen rich areas such as Nilgiri and Palni Hills, Eastern Ghats, and major portion of Western Ghats, in Tamil Nadu is the reason for lichen richness in the state. Major part of West Bengal state for instance comes under Gangetic plains. Darjeeling district of West Bengal actually lays in Eastern Himalayas and has high diversity of lichens. Gujarat, Mizoram and Puducherry are represented by 2 species each, Punjab by 3 species, Bihar and Chattisgarh by 6 species each, Lakshadweep by 9 and Jharkhand by 10 species only[6].

Lichens synthesize many useful secondary metabolites which are antiviral, anti-bacterial, antitumor, anti-allergic and have an inhibitory effect on the growth of plants[7,8,9]. The use of lichens in medicine is due to their secondary metabolites that are unique compared to those of higher plants[10]. These different metabolites along with other chemical compounds can be utilized for curing aches and diseases. While numerous activities of lichen metabolites are now recognized, their therapeutic potential has not been fully explored and remains pharmaceutically unexploited[11]. The use of lichens in medicine is based on the fact that they contain unique, relatively low molecular weight and varied biologically active substances[12]. It has folkloric repute of cosmetics for skin bleach and has been prescribed to for the management of diarrhoea, dyspepsia, spermatorrhoea, amenorrhea, dysentery and wound healing[13,14]. Lichens have economic benefits to human beings which has antibiotic properties that are valuable commercially for biomedical applications. Lichens and their metabolites yield significant bioactive substances for the treatment of various human diseases caused by different pathogenic microorganisms[15].

New studies have revealed that these slow-growing organisms produce a diverse array of secondary metabolites with different biological activities[16]. The biological potential of many lichens and their metabolites has largely remained unexplored. Thus, the aim of the present work was to identify the secondary metabolites of *Hypotrachyna infirma by* GC-MS.

MATERIAL AND METHODS

Collection of Plant Material

Hypotrachyna infirma (Figure No.1) was collected from Sholaiyar, Anamalai hills, Coimbatore district, Tamilnadu, India during the monsoon season i.e. July 19, 2019. The lichen is duly identified by Dr. Sanjeeva Nayaka, Senior Principal Scientist, Lichenology Laboratory, CSIR-National Botanical Research Institute, Lucknow, India and was deposited at herbarium of LWG with Accession No.36008.

Preparation of extract

For extraction, lichen sample was shade dried for one to two weeks. After drying, it was grinded and stored in an airtight container. The air dried lichen powdered material (10g) was transferred into 250ml quick fit flask and extracted in the soxhlet extractor for 48 hours[17,18] using different organic solvents such as Ethyl acetate, Chloroform, Ethanol, Water and Methanol. The extracts were filtered over Whatman No.1 filter paper and the filtrates were concentrated under reduced pressure to pasty mass[19] for further studies.

Extraction Yield (%)

The yield of the extracts was calculated by using the following formula:

$$\text{Yield (\%)} = \frac{\text{Total crude extract (g)}}{\text{Dried powder (g)}} \times 100$$

Among all the extracts, methanol extract showed comparatively better yield than Ethyl acetate, Chloroform, Ethanol and Water. So methanol extract was used for GC-MS analysis.

Gas chromatography-MS analysis

GC-MS analysis was performed at the Indian Institute of Crop Processing Technology, Thanjavur, Tamilnadu, India. Five ml of methanol extract was evaporated to dryness and reconstituted in 2ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was accomplished with CE GC 8000 top MSMD 8000 Fyson instrument with Db 35mr column (10m x 0.5mm, 0.25mm film thickness). Heating programs were executed from 100-250ºC at 3 minutes by using helium as a carrier gas with a flow rate of 1ml/min in the split mode (1:50). An aliquot (2ml) of oil was injected into the column with the injector heater at 250ºC.

Analytical conditions

Injection temperature at 250ºC, interface temperature at 200ºC, quadruple temperature at 150ºC and ion source temperature at 230ºC were maintained. Injection was performed in split less mode.

Identification of components (Data analysis)

The mass spectra of compounds in samples were attained by electron ionization (El) at 70 eV. In Scan mode the detector operated from 20 to 600 atomic mass units (amu). Identifications were based on the molecular structure, molecular mass and calculated fragmentations. Resolved spectra were identified for phytochemicals by using the standard mass spectral database of WILEY and NIST[20,21].

Identification of components

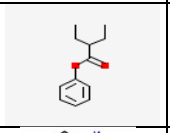
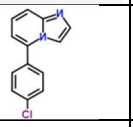
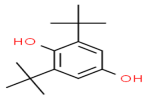
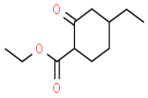
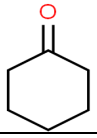
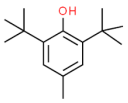
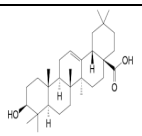
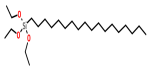
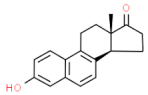
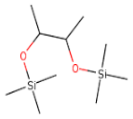
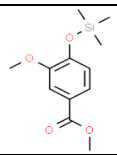
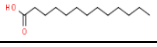
Based on the molecular structure, molecular mass and calculated fragments, the identification have been done. Analysis on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST), which have more than 62,000 patterns. The compound name, molecular weight and their structure of the components of the test materials were ascertained. The average peak area to the total areas of the component was compared to calculate the relative percentage amount of each component. By using the NIST library version (2005), software, Turbomas 5.2, the spectrum of the unknown component was compared with the spectrum of the component stored in the software.

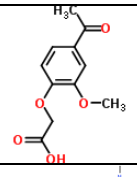
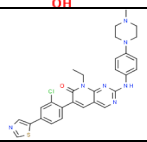
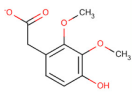
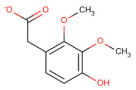
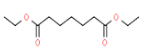
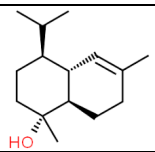
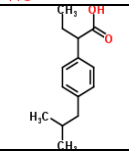
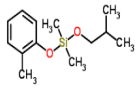
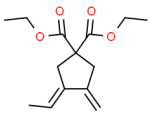
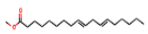
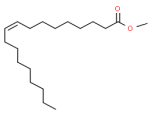
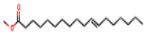
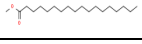
RESULTS AND DISCUSSION

GC-MS is a best technique to identify the constituents of volatile matter, long chain, branched chain hydrocarbons, alcohols, acids, esters, etc., Peak area; retention time and molecular formula are used for the confirmation of phytochemical compounds[22]. The mass spectrometer analyzes the compounds rinsed at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to arrival of peaks at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the Wiley Spectral library[23]. Applications of GC-MS include drug detection, environmental analysis, explosives investigation, and identification of unknown samples[24]. Gas chromatography is attached to a Mass Spectrometer (GC-MS) enables mixture of small molecules mainly organic compounds of low molecular weight (<600) which can be analyzed[25].

GC-MS chromatogram of the methanolic extract of *Hypotrachyna infirma* (Figure No.2) showed forty peaks indicating the presence of forty compounds[26]. These compounds were identified through mass spectrometry attached with GC. The active principles with their retention time (RT), molecular formula, molecular weight and structure are presented in Table No.1. Phytochemical analysis by GC-MS analysis of the methanolic extract of *Hypotrachyna infirma* revealed the presence of different fatty acids, heterocyclic compounds, and esters among others. The results obtained from this study represented an important step towards the effective characterization of the secondary metabolite compounds from this lichen using GC-MS analysis. The lichen could be used for the management of various health related issues associated with the metabolites screened.

Table No.1: Chemical constituents of methanolic extract of *Hypotrachyna infirma* (kurok.) Hale

S.No	RT	Name of The Compound	Molecular Formula	Molecular Weight G/Mol	Structure	Peak %
1	5.620	2-Ethylbutyric acid, hexadecyl ester	$C_{24}H_{48}O_2$	368.6		0.43
2	5.731	2-(4-Chlorophenyl)-5, 7-dimethylimidazo[1, 2-a]pyridine-8-carbonitrile	$C_{13}H_9ClN_2$	228.67		0.30
3	6.564	1, 2-Benzenediol, 3, 5-bis(1, 1-dimethylethyl)-	$C_{14}H_{22}O_2$	222.32		1.55
4	8.009	Cyclopentanecarboxylic acid, 2-oxo-, methyl ester	$C_{11}H_{18}O_3$	198.26		6.20
5	8.820	Azulene	$C_{10}H_8$	128.17		0.89
6	9.064	Nickel, (.eta.-4-diallyl ether)-(2, 4-dimethyl-3-pentylisonitrile)	$C_6H_{10}O$	98.14		1.80
7	9.175	Tricyclo[5.2.2.0 (2, 6)]undec-8-en-11-one, 3-[(2-methoxyethoxy) methoxy]-2-methyl-	$C_{15}H_{24}O$	220.35		0.53
8	9.742	22.beta.-Acetoxy-3.beta., 16.alpha.-dihydroxy-13, 28-epoxyolean-29-al	$C_{32}H_{50}O_6$	530.7		1.04
9	11.475	Octadecyltriethoxysilane	$C_{24}H_{52}O_3Si$	416.8		0.34
10	11.542	Benzenepropanoic acid, 3-phenyl-2-propenyl ester	$C_{18}H_{18}O_2$	266.3		0.32
11	12.564	Butane, 2, 3-bis (trimethylsiloxy)-	$C_{10}H_{26}O_2Si_2$	234.48		0.34
12	12.786	Methyl 2-hydroxy-4-methoxybenzoate, trimethylsilyl ether	$C_{12}H_{18}O_4Si$	254.35		0.44
13	13.430	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	214.34		22.12

14	15.208	Benzoic acid, 3-formyl-2,4-dihydroxy-6-methyl-, methyl ester	$C_{11}H_{12}O_5$	224.21		1.56
15	15.308	Pyrido[2,3-d]pyrimidin-5(8H)-one, 4-methyl-2-phenyl-	$C_{29}H_{28}ClN_7OS$	558.1		0.66
16	15.619	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	$C_{10}H_{12}O_4$	196.2		27.34
17	15.763	Benzoic acid, 2,4-dihydroxy-3,6-dimethyl-, methyl ester	$C_{10}H_{12}O_4$	196.2		1.29
18	16.797	Adipic acid, butyl methyl ester	$C_{11}H_{20}O_4$	216.27		2.37
19	16.952	3, 3, 7, 11-Tetramethyltricyclo [5.4.0.0(4, 11)]undecan-1-ol	$C_{15}H_{26}O$	222.37		0.66
20	17.108	Motrin methyl eSster	$C_{14}H_{20}O_2$	220.31		0.40
21	17.174	Trimethyl[4-(1-methyl-1-methoxyethyl) phenoxy] silane	$C_{13}H_{22}O_2Si$	238.4		0.46
22	17.863	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.5		10.96
23	18.807	Fumaric acid, di (non-5-yn-3-yl) ester	$C_{14}H_{20}O_4$	252.31		0.90
24	19.496	9,12-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	310.5		1.28
25	19.552	9-Octadecenoic acid, methyl ester, (E)-	$C_{19}H_{36}O_2$	296.5		3.76
26	19.607	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296.5		0.52
27	19.785	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298.5		5.90

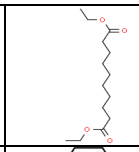
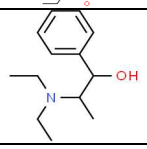
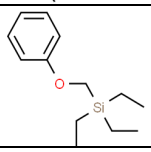
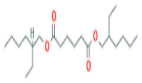
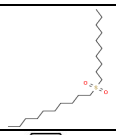
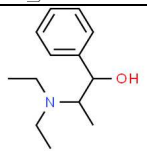
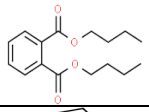
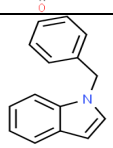
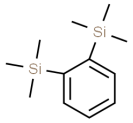
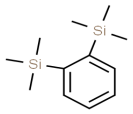
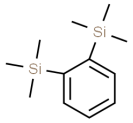
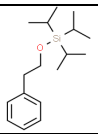
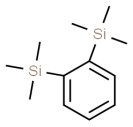
28	20.007	Hexanedioic acid, mono(2-ethylhexyl) ester	$C_{14}H_{26}O_4$	258.35		0.50
29.	21.318	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31		0.27
30	21.540	Silane, trimethyl[5-methyl-2-(1-methylethyl) phenoxy]-	$C_{13}H_{22}OSi$	222.4		0.30
31	22.051	Hexanedioic acid, bis (2-ethylhexyl) ester	$C_{22}H_{42}O_4$	370.6		0.40
32	22.940	Di-n-decylsulfone	$C_{20}H_{42}O_2S$	346.6		0.25
33	23.062	N-Methyl-1-adamantaneacetamide	$C_{13}H_{21}NO$	207.31		0.76
34	23.207	1, 2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278.34		1.07
35	23.273	Benzo[h]quinoline, 2, 4-dimethyl-	$C_{15}H_{13}N$	207.27		0.29
36	23.718	1, 2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47		0.25
37	24.284	1, 2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47		0.60
38	24.451	Silane, 1, 4-phenylenebis[trimethyl-	$C_{12}H_{22}Si_2$	222.47		0.25
39	24.740	Trimethyl[4-(1, 1, 3, 3,-tetramethylbutyl) phenoxy]silane	$C_{17}H_{30}OSi$	278.5		0.31
40	25.962	1, 2-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222.47		0.39

Figure No.1: Habit of *Hypotrachyna infirma*

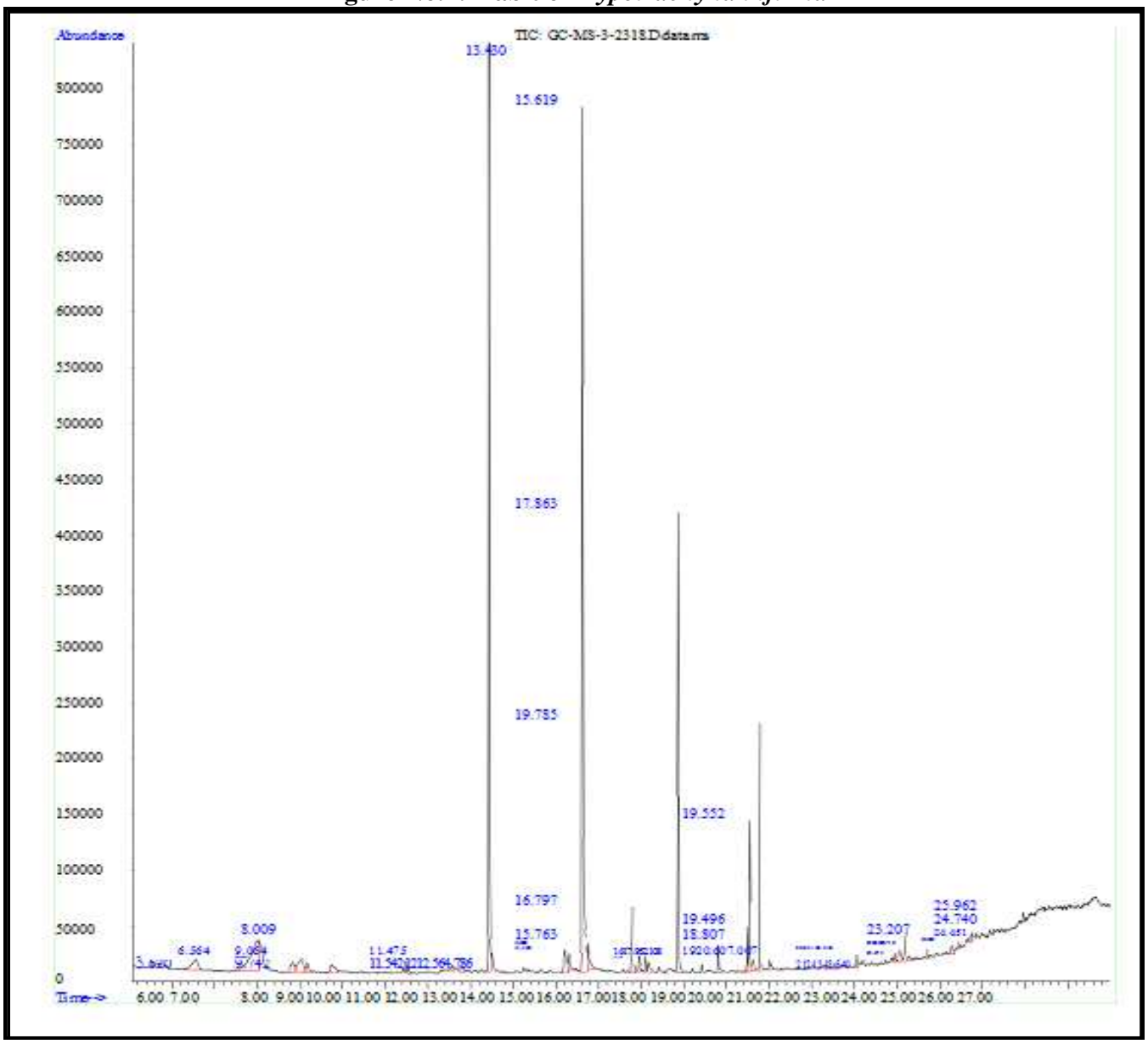


Figure No.2: GC-MS Analysis of *Hypotrachyna infirma*

CONCLUSION

In the present study, the presence of various bio-active compounds was detected, after the GC-MS analysis using methanolic extract of *Hypotrachyna infirma.* Isolation of new phytocompounds from this lichen (*Hypotrachyna infirma*) and their biological activity will ensure best possible results in future and open new opportunity for discovery of potential drugs. However, isolation of individual phytochemical constituents will be definitely giving fruitful results and will open a new area of investigation of individual components and their pharmacological potency.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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