Educational Administration: Theory and Practice
2024, 30(2), 1418 - 1423
ISSN: 2148-2403
https://kuey.net/

Research Article

GC-MS Analysis Of The Polyherbal Mixture of *Parmotrema tinctorum*, *Waltheria Indica* and *Frageria vesca*

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Citation: Meraj Sultana, Dr. Ch. Kantlam (2024), GC-MS Analysis Of The Polyherbal Mixture, *Educational Administration: Theory and Practice*, 30(2), 1418 – 1423
Doi: 10.53555/kuey.v30i2.7309

ARTICLE INFO

ABSTRACT

The study is unique in its focus on the phytoconstituents of the chloroform extract of a polyherbal mixture, which contains lichens of Parmotrematinctorum, aerial parts of WaltheriaIndica and Frageriavesca. The investigation is conducted using GC-MS (gas chromatography-mass spectrometry). The GC-MS analysis of the polyherbal mixture is performed using Agilent 6890 GC with 59739N MSD and GC-MS equipped with Elite-I fused with silica capillary column (Cpsil 8cb: 30mm x 25mm x 0.25mm). The result of the analysis confirms the presence of 61 compounds, with the most prevailing compounds of medicinal value being isophytol, heneicosane, hentriacontane, aziridine, 3 eicosene, atranol, neophytadiene, phytoplankton,3-eicosane, anthracene,phenanthridine,squalene, etc.

Keywords: Polyherbal mixture, lichens of *Parmotrema tinctorum*, *Waltheria Indica* and *Frageria vesca*, GC-MS Analysis.

I. INTRODUCTION

The use of plants as a source of medicine has been inherited and is an essential component of the health care system. India is the substantial producer of medicinal herbs and is thus aptly called the "botanical garden of the world.[1]

Parmotrematinctorum, belonging to the Parmeliaceaefamily, is commonly known as "patherkephool" or "bojhwar" and is utilized as a fungal spice in India [2]. This lichen is predominantly found growing on trees and rocks. Chemical components such as praesorediosic acid, protocentratric acid, usnic acid, lecanoric acid, orsenillic acid, lichenin, and iso lichenin are identified within Parmotrematinctorum. It serves as a spice to enhance the flavour of dishes. It possesses various medicinal properties, including anti-toxin, antipyretic, anti-inflammatory, and analgesic effects, and aids in treating motion sickness and heart problems. *Parmotrema tinctorum* finds applications in Indian medicine, Chinese medicine, and homeopathy [2].

Waltheria indica, a member of the Sterculiaceaefamily, is commonly referred to as velvet leaf, sleepy morning, or marshmallow in English and known as "shengalipoondu" in Telugu. This plant is native to subtropical and tropical regions. It is predominantly found in shrub forests, grasslands, rocky hills, and soils across East and West Africa and South America, as well as in various regions of India such as Punjab, Assam, Odisha, Andhra Pradesh, and Kerala . Chemical analysis of W. indica reveals the presence of flavonoids, tannins, glycosides, saponins, carbohydrates, alkaloids, triterpenes, and anthraquinones. All parts of the plant are used to treat coughs, eye irritations, ulcers, neurological conditions, and lung infections and to combat malaria, fever, typhoid, and fatigue [3].

Fragaria vesca, a member of the Rosaceae family, is commonly known as wild strawberry or woodland strawberry, and it bears small, sweet, red berries.Native to Europe, it thrives in woodland environments[4].Fragariavesca contains various chemical components, including anthocyanins, flavonoids, tannins, ellagic acid, terpenoids, sugars, vitamins, fibres, and organic acids. In some cultures, such as certain

Native American traditions, it holds symbolic significance associated with love and friendship and is utilized in ceremonial practices. Additionally, it is employed to impart flavour to beverages like mead and wine and as a natural dye for textiles and baskets.[4] The constituents of Fragariavesca are attributed to pharmacological properties such as anti-inflammatory, antimicrobial, anticancer, antidiabetic, cardiovascular disorder treatment, and diuretic effects. [5][6]

The investigation has shown that there are no published reports worldwide related to the possible chemical components in the mixture. So, the present study aimed to investigate the possible chemical components by first preparing the chloroform extract and identifying the compounds by subjecting them to GC-MS analysis..

II. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Material

*Parmotrematinctorumlichens*were gathered from an Ayurvedic shop in Hyderabad and verified by P.V. Prasanna of the Botanical Survey of India, Atapur, Hyderabad, with identification number BSI/DRC/2019_20/Tech/identification/362.

The aerial parts of Waltheriaindica and Fragariavesca were Gathered from Chittoor District and verified by Dr. K. Madhava Chetty, a highly respected Plant Taxonomist and Assistant Professor in the Department of Botany at Sri Venkateshwara University, Tirupati. His expertise and experience in plant taxonomy ensure the accuracy of the plant identification process, a crucial step in this study.

2.2 Extraction 300gm of dried plant materials (1:1:1) was meticulously extracted with chloroform by maceration. The extract/mixture thus obtained was subjected to evaporation on water bath until it becomes semisolid, then was stored in airtight container for further use. This careful extraction process ensures the purity and integrity of the sample for accurate analysis.

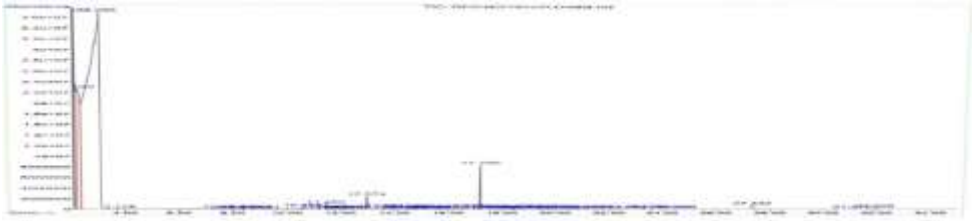
2.3 GC-MS (Gas Chromatograhy-Mass Spectrometry) Analysis GC-MS analysis of polyherbal mixture was performed by using Agilent 6890 GC with 59739N MSD and G.C. -M.S. equipped with Elite –I, fused with silica capillary column (cpsil 8cb:30 mm x 25mm x 0.25 mm) composed of 100% Dimethylpolysiloxane. For GC-MS detection, an electron ionization system with an ionizing energy of 70 E.V. was used. Hydrogen (99.99%) was used as the carrier gas at a flow rate of 1.5 ml and an injection volume of 2 microlitres. It was employed with a split ratio 10:1, initial injection temp of 400 C to final 2800 C, ion source temp 2300 C. The oven temp was programmed from 1100 C (isothermal for 2 minutes) with a rate of 100 C per minute to 300 C, a scan interval of 0.5 sec, and fragments from 20-200 Dalton. Total G.C. running time was 36 minutes. The relative average peak area and retention time, as well as the molecular formula with that of molecular weight, were obtained. Mass spectrum GC-MS was interpreted using the National Institute Standard and Technology (NIST) database, having 62,000 patterns. The spectrum of unknown components was compared of those of known constituents that were stored in the NIST library. The name, and the molecular weight and structure of the components of the sample material were identified.

III. RESULTS

3.1 Extraction The % yield of chloroform extract of polyherbal mixture was found to be 6.27%.

3.2 GC-MS (Gas Chromatograhy-Mass Spectrometry) Analysis

GC-MS spectrum of chloroformextract of polyherbal mixture containing lichens of Parmotrematinctorum*WaltheriaIndica* and *Frageriavesca*is shown in Graph 1. The GC-MS analysis of polyherbal mixture revealed 61 phytochemical constituents namely, Azotensin; Thymol; Erythrocentaurin; Neophytadiene; Hexadecanoic acid; Cis-9, Cis-12-Octadecadienoic acid, Squalene which may contribute to the medicinal activity of the extract. The major phytochemical constituents identified by GC-MS with their biological activity are presented in TABLE 1.



Graph 1: GC-MS Spectrum of Chloroform extract of Polyherbal Mixture

S.No.	Name	RT (Min)	Mol. Formula	M.W (g/mol)	Peak Area %	Biological Activity
1	Trichloromethane	2.160	$CHCl_3$	119.38	7.339%	Anaesthetic
2	2-Pyrrolidinone	2.249	C_4H_7NO	85.106	13.14 %	Antimicrobial
3	Methane, oxybisdichloro	3.086	$C_2H_2Cl_4O$	183.849	76.47%	Antimicrobial
4	Toluene	3.724	$C_6H_5CH_3$	92.141	0.05%	Neurotoxic, hepatotoxic, nephrotoxic, reproductive toxicant.
5	Decane	7.427	$C_{10}H_{22}$	142.28	0.03%	Mild irritant, low acute toxicity
6	Hexane 3,3-dimethyl	7.660	C_8H_{18}	114.2285	0.01%	Neurotoxic, reproductive toxicant.
7	Decane, 3,7-dimethyl	8.389	$C_{12}H_{26}$	170.33	0.03%	mild irritants
8	Ethanone 1-cyclopentyl	8.749	$C_7H_{12}O$	112.1696	0.01%	CNS depressant
9	2,3-Dimethyl-3-heptene	8.813	C_9H_{18}	126.24	0.01%	Potential Irritant
10	3-Ethyl-3-methylheptane	9.101	$C_{10}H_{22}$	142.2817	0.01%	Antimicrobial
11	Dodecane	10.586	$C_{12}H_{26}$	170.340	0.05%	antibacterial activity and antifungal activity.
12	2,6,10-Trimethyltridecane	11.245	$C_{16}H_{34}$	226.441	0.01%	antifungal, antibacterial and nematicidal
13	1,3-bis(1,1-dimethylethyl	11.450	$C_{14}H_{22}$	190.3245	0.11%	anti-bacterial activity
14	Undecane2,4-dimethyl-	11.518	$C_{13}H_{28}$	184.36	0.01%	fungicidal
15	l-Threitol, 2-O-nonyl-	11.756	$C_{13}H_{28}O_4$	248.36	0.02%	alkylating agent
16	Oxalic acid, 6-ethyloct-3-yl hexyl	12.131	$C_{18}H_{34}O_4$	314.5	0.02%	anti-inflammatory, antibacterial, antioxidant, anthelmintic, antidiabetic, anticancer, mosquitocidal, and insecticidal activity
17	4-Isopropyl-1,3-cyclohexanedione	12.245	$C_9H_{14}O_2$	154.21	0.02%	Antimicrobial
18	Octane, 5-ethyl-2-methyl-	12.381	$C_{11}H_{24}$	156.31	0.03%	Antimicrobial
19	3,5-Dihydroxytoluene	12.974	$C_7H_8O_2$	124.14	0.43%	biological dye and indicator
20	Tetradecane	13.362	$C_{14}H_{30}$	198.38	0.04%	Antimicrobial
21	2,5-Dimethylhydroquinone	13.651	$C_8H_{10}O_2$	138.16	0.06%	Antimicrobial
22	Heneicosane	14.179	$C_{21}H_{44}$	296.58	0.01%	Antimicrobial
23	Sulfurous acid, 2-ethylhexyl non	14.416	$C_{14}H_{30}O_3S$	278.45	0.01%	antioxidant and antibacterial activity
24	n-Eicosane	14.528	$C_{20}H_{42}$	282.56	0.01%	antifungal activity

25	Hexacosane, 1-iodo	14.607	$C_{26}H_{53}I$	492.6	0.04%	antibacterial activity
26	Phenol, 2,5-bis(1,1-dimethylethyl)-	14.821	$C_{14}H_{22}O$	206.32	0.03%	anti-bacterial activity, anti-fungal activity and antioxidant , antiinflammatory activity and anti-cancer activity
27	1-Dodecanol, 2-hexyl-	15.072	$C_{18}H_{38}O$	270.49	0.02%	Antimicrobial Activity
28	10-Methylnonadecane	15.152	$C_{20}H_{42}$	282.55	0.03%	antibacterial activity
29	2,3-Dimethyl-3-heptene	15.284	C_9H_{18}	126.24	0.01%	Antimicrobial Activity
30	Atranol	15.340	$C_8H_8O_3$	152.15	0.01%	antibacterial activity
31	Tetradecane, 3-methyl	15.381	$C_{15}H_{32}$	212.41	0.02%	antibacterial and antifungal
32	2-Azido-5-methylbenzylamine	15.669	$C_8H_{11}N$	121.18	0.02%	analgesic,
33	1,5,5-Trimethyl-6-methylene-cyclo	16.633	$C_{10}H_{16}$	136.23	0.03%	antibacterial and antioxidant activities
34	Heptadecane	16.968	$C_{17}H_{36}$	240.47	0.04%	ibacterial activity
35	Tetracosane	17.120	$C_{24}H_{50}$	338.65	0.01%	antibacterial, antidiabetic, and antitumor activities, antioxidant and antimicrobial activities.
36	Benzoic acid, 2,4-dihydroxy-3,	17.190	$C_{10}H_{12}O_4$	154.12	0.10%	antibacterial activity
37	2-Bromo dodecane	17.583	$C_{12}H_{25}Br$	249.23	0.02%	alcohol-antimicrobial, insecticidal activity
38	Decane, 3,8-dimethyl-	17.837	$C_{12}H_{26}$	170.33	0.01%	antimicrobial activity
39	E-15-Heptadecenal	17.977	$C_{17}H_{32}O$	252.4	0.01%	anti-inflammatory, hematopoietic, osteoclastogenic, neurogenic
40	Disulfide, di-tert-dodecyl	18.096	$C_{24}H_{50}S_2$	402.78	0.01%	insecticide and fungicide
41	Tetramethrin	18.401	$C_{19}H_{25}NO_4$	331.4	0.02%	Insecticide
42	Neophytadiene	18.478	$C_{20}H_{38}$	278.51	0.02%	Anti-inflammatory, Antimicrobial, Antioxidant
43	Benzene, (propylthio)-	18.739	$C_{10}H_{14}S$	166.29	0.02%	anticancer activity
44	Isophytol	18.919	$C_{20}H_{40}O$	296.53	0.01%	antimicrobial activity
45	Heneicosane	19.360	$C_{21}H_{44}$	296.58	0.04%	antimicrobial activity
46	Hentriacontane	19.660	$C_{31}H_{64}$	436.83	0.01%	antipyretic, antitumor, hypotensive, antianaphylactic, antiasthmatic, and aphrodisiac
47	Aziridine	19.763	C_2H_5N	43.07	0.05%	Anticancer Agent
48	3-Eicosene	20.004	$C_{20}H_{40}$	280.5	0.01%	antimicrobial, antihyperglycemic,

						cytotoxic, antioxidant and insecticidal
49	Hexadecanal	20.290	$C_{16}H_{32}O$	240.43	0.01%	Antioxidant and antibacterial
50	Octadecanenitrile	21.103	$C_{18}H_{35}N$	265.47	0.02%	Solvent, a lubricant, transformer oil and an anti-corrosion agent
51	Phytol	21.172	$C_{20}H_{40}O$	296.53	0.02%	anxiolytic, metabolism-modulating, cytotoxic, antioxidant, autophagy- and apoptosis-inducing, antinociceptive, anti-inflammatory, immune-modulating, and antimicrobial effects.
52	Eicosane	21.392	$C_{20}H_{42}$	576.76	0.02%	antifungal activity
53	Dotriacontane, 1-iodo-	21.761	$C_{32}H_{65}I$	282.56	0.01%	antibacterial activity
54	Anthracene, 9-(3-bromopropyl)-9	22.682	$C_{17}H_{15}Br$	299.21	0.01%	anticancer, antitumor, antiinflammatory, antiarthritic, antifungal, antibacterial, and antimalarial
55	Phenanthridine	23.292	$C_{13}H_9N$	179.21	0.03%	antitubercular activity
56	Hentriacontane	23.577	$C_{31}H_{64}$	436.83	0.02%	antipyretic, antitumor, hypotensive, antianaphylactic, antiasthmatic, and aphrodisiac
57	Tetratriacontane	24.382	$C_{20}H_{42}$	282.5	0.01%	antibacterial and antitumor
58	1,3-dimethylguanine	24.533	$C_7H_{11}N_5$	181.19	0.01%	Antiviral Activity
59	Squalene	27.332	$C_{30}H_{50}$	410.73	0.15%	Antioxidant Moisturizing Cholesterol Biosynthesis Precursor Anti-inflammatory Anticancer Potential
60	Isotridecanol	31.076	$C_{13}H_{28}O$	200.36	0.06%	Surfactant Low Toxicity Skin and Eye Irritant Biodegradable
61	Vitamin E	31.909	$C_{29}H_{50}O_2$	430.70	0.11%	Antioxidant Anti-inflammatory Skin Health Cardiovascular Health Neurological Function

IV. CONCLUSION

The presence of different types of bio-active compounds detected after GC-MS analysis using the chloroform extract of a polyherbal mixture containing lichens of ParmotrematinctorumWaltheriaIndica and Frageriavesica justifies the use of the whole plant for various elements by traditional practitioners. However, isolating individual phytochemical constituents and subjecting them to biological activity will yield fruitful results. It will open a new area of investigation of individual components and their pharmacological potency. From these results, it could be concluded that chloroform extract of a polyherbal mixture containing lichens of Parmotrematinctorum, WaltheriaIndica and Frageriavesica contains various bio-active compounds which have varied pharmacological activities for which study has to be done. Therefore, it is endorsed as a plant of phytopharmaceutical importance.

ACKNOWLEDGEMENT

The authors may well like to express their profound gratitude to Dr. Vijaya Sarathi, Sr. Scientist at IICT, Tarnaka, Hyderabad, Telangana, India. His provision of facilities and support was instrumental in the successful execution of this research work.

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