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SPECTRAL ANALYSIS OF PETROLEUM ETHER AERIAL PART EXTRACT OF *GRANGEA MADERASPATANA* USING GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Keywords:

Grangea maderaspatana, Phytochemicals, GC-MS, Soxhlet extraction, Methanol extract

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ABSTRACT: *Grangea maderaspatana*, commonly known as Madras carpet / Indian chickweed and belonging to the Asteraceae family. This plant is widely distributed throughout the tropical and subtropical regions of Asia and Africa and traditionally employed in the management of respiratory, gastrointestinal and dermatological disorders. *Grangea maderaspatana* plant has also demonstrated potential anti-inflammatory, analgesic and antimicrobial activities. This study investigates the chemical composition of the petroleum ether extract of aerial part of *Grangea maderaspatana* using Gas Chromatography-Mass Spectrometry (GC-MS). The plant, known for its traditional medicinal uses, was subjected to Soxhlet extraction and the extract was analyzed to identify its phytochemical components. The analysis revealed a complex mixture of hydrocarbons, fatty acids, terpenes and other organic compounds with tetracontane (45.20% area at different retention time), n-hexadecanoic acid (7.05%area), (S,Z)-heptadeca-1,9-dien-4,6-diyn-3-ol (6.18% area at different R. Time) and neophytadiene (5.08%area) being the major components. The presence of these compounds suggests potential applications in pharmaceuticals, cosmetics and other industries due to their bioactive properties.

INTRODUCTION: *Grangea maderaspatana*, commonly known as Madras carpet or Indian chickweed, is a prostrate annual herb belonging to the Asteraceae family. This plant is widely distributed across tropical and subtropical regions of Asia and Africa, particularly in India, Sri Lanka, and parts of Southeast Asia. *G. maderaspatana* has garnered significant scientific interest due to its traditional medicinal uses and potential pharmacological properties. Morphologically, *G. maderaspatana* is characterized by its spreading habit, with stems reaching up to 30-45 cm in length. The leaves are simple, alternate, and deeply lobed, giving the plant a distinctive appearance.

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The inflorescence consists of small, yellow flower heads that are solitary or clustered at the ends of branches [1]. Ethnopharmacological studies have reported various traditional uses of *G. maderaspatana* in folk medicine. It has been employed in the treatment of various ailments, including respiratory disorders, gastrointestinal issues, and skin diseases. The plant is also known for its potential anti-inflammatory, analgesic, and antimicrobial properties [2].

Phytochemical investigations have revealed the presence of diverse bioactive compounds in *G. maderaspatana*, including flavonoids, terpenoids, and phenolic compounds. These secondary metabolites are believed to be responsible for the plant's medicinal properties and have been the subject of numerous scientific studies aiming to elucidate their structures and biological activities [3]. Recent pharmacological studies have focused on evaluating the therapeutic potential of *G. maderaspatana* extracts and isolated compounds.

Research has shown promising results in areas such as hepatoprotection, antioxidant activity, and anti-diabetic effects, suggesting that this plant may have significant potential in modern medicine and drug discovery [4].

MATERIALS AND METHODS:

Plant Material: The air-dried aerial parts 1.5 kg, purchased from local vendor of Jaipur Rajasthan India.

Extraction: Using a grinder, the air-dried plant material was first reduced to a fine powder. Then, 300 ml of petroleum ether solvent were used to perform a Soxhlet extraction technique on 100 gm of the powdered material for 12 hours X 3 days. A rotary vacuum evaporator (make: N.N. Series from Eyela, Tokyo, Japan) was used to remove extra solvent along with a digital water bath SB-651. The extract was treated with sodium sulphate to remove any moisture that might have remained. A concentrated sample of the extract was obtained after it was filtered to remove any grainy material using Whatman No. 1 filter paper. The final extract was kept in an airtight container at low temperature.

GC-MS Analysis: Gas Chromatography combined with Mass Spectrometry is a preferred methodology for routine analysis of compounds. The GC-MS analysis of the above-mentioned extracts was performed with a Gas Chromatography unit Shimadzu GCMS-QP2010 Plus comprising AOC-20i+s auto sampler at Manipal University Jaipur Rajasthan India. Various components were identified by different retention times, which were detected by a mass spectrophotometer. The chromatogram plot of intensity against retention time was recorded by the software attached to it. From the graph, the compounds are identified, comparing the data with the existing software libraries like WILEY8.lib, NIST11.lib, NIST11s.lib, FFNSC2.lib and mass spectra of standard. The name, molecular weight, and structure of the components of the test materials were ascertained.

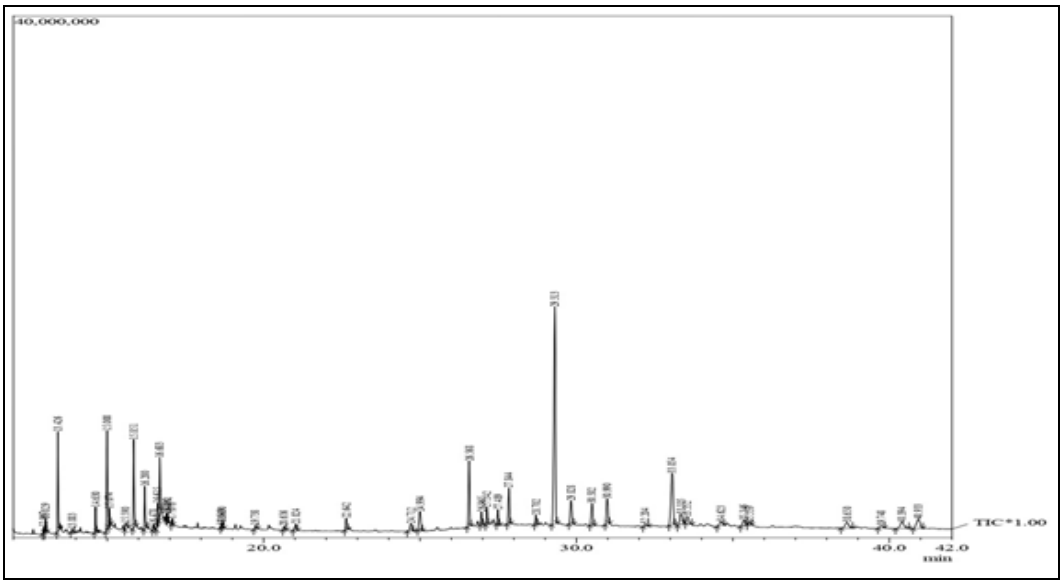
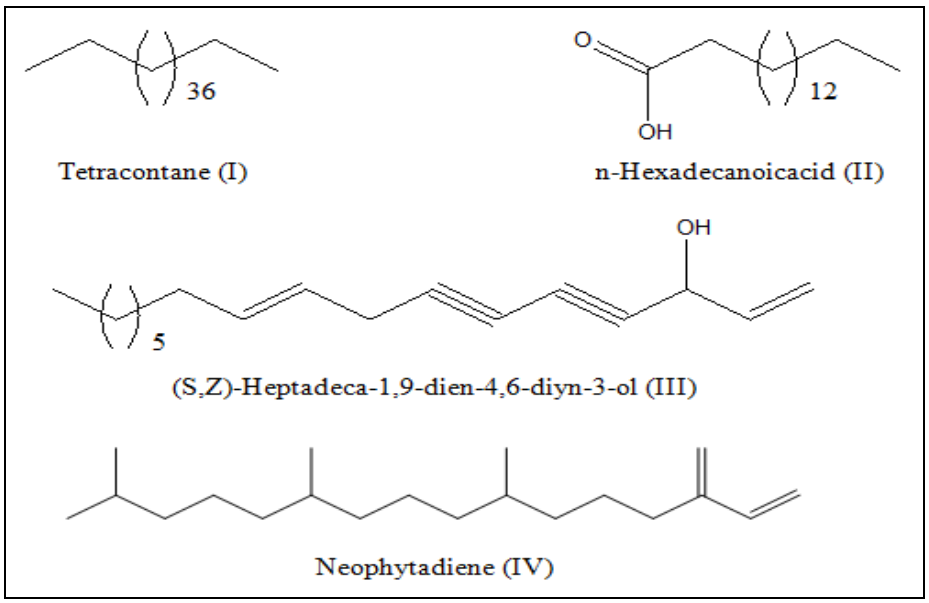


FIG. 1: GC-MS SPECTRUM OF PETROLEUM ETHER AERIAL PART EXTRACT OF *G. MADERASPATANA*

RESULTS AND DISCUSSION: The most abundant compound detected in the extract of *G. maderaspatana* is tetracontane with 45.20% area at different retention time. It is a long-chain alkane having 40 carbon atoms in the chemical structure. Similarly n-hexadecanoic acid (palmitic acid) with 7.05% area also found in the extract which is a common saturated fatty acid found in plants and animals. Other significant components include (S,Z)-heptadeca-1,9-dien-4,6-diyn-3-ol (6.18% area at different R. Time), an unusual unsaturated alcohol and neophytadiene (5.08% area), a diterpene found in the petroleum ether aerial part extract of *G. maderaspatana*. Generally, neophytadiene found in tobacco plants. In this extract, phytol (2.66%), various fatty acids such as 9, 12, 15-octadecatrienoic acid (3.84%), and squalene (2.43%) sterols like chondrillasterol

(1.87%) and alpha-amyrin (0.86%) are also detected. The mixture contains a range of hydrocarbons, fatty acids, terpenes and other organic compounds, indicating a complex natural product.



Tetracontane has found diverse applications across different industries due to its inimitable properties. In the field of materials science, tetracontane has been investigated as a phase change material for thermal energy storage applications. It's high latent heat of fusion and suitable melting temperature range, make it a potential molecule for use in building materials to improve energy efficiency [5]. In the pharmaceutical industry, tetracontane has been studied as a potential excipient in drug formulations, particularly for controlled release applications. It's hydrophobic nature can be utilized to modulate drug release profiles in certain dosage forms [6]. In the cosmetics industry, tetracontane is used in formulations for their emollient properties and ability to form protective barriers on the skin [7].

Additionally, tetracontane used as a reference compound in gas chromatography to calibrate retention times and identify unknown compounds in complex mixtures [8]. It's presence in natural waxes also makes it relevant in the study of plant physiology and ecology, particularly in understanding plant adaptations to water stress and environmental conditions [9]. n-Hexadecanoic acid, also known as palmitic acid, is a saturated fatty acid with diverse applications across multiple industries. In the pharmaceutical sector, it serves as an excipient in drug formulations, enhancing drug solubility and absorption [10]. The cosmetics industry widely uses n-hexadecanoic acid in skincare products for its emollient properties and ability to form a protective barrier on the skin [11]. In food science, it is a common ingredient in food additives and used as a flavoring agent [12]. n-Hexadecanoic acid is utilized in the production of soaps, lubricants and plasticizers [13] and it plays a important role in cellular metabolism and serves as a precursor for longer fatty acids [14]. Recent research has also explored it's therapeutic potential as an antimicrobial and anti-inflammatory agent [15].

(S,Z)-Heptadeca-1, 9-dien-4, 6-diyn-3-ol is a unsaturated alcohol belongs to the class of polyacetylenic compounds, which is often found in plants of the Asteraceae family and have attracted interest due to their potential biological activities. Studies on related polyacetylenic alcohols compounds have demonstrated anti-inflammatory, antimicrobial and cytotoxic activities, suggesting potential applications in drug discovery and development [16]. In natural product chemistry, the presence of such compounds can serve as chemotaxonomic markers, aiding in the classification and identification of plant species [17, 18]. Neophytadiene is a diterpene hydrocarbon, often used as a biomarker for certain plant species, particularly in tobacco and some marine algae [19]. Studies have shown that neophytadiene exhibits

antimicrobial properties, demonstrating effectiveness against certain bacterial and fungal strains [20]. Neophytadiene investigated for its potential anti-inflammatory and analgesic effects suggesting possible applications in pain relief management and inflammatory conditions [21]. It's antioxidant properties, indicating potential use in preventing oxidative stress-related diseases [22]. In the fragrance industry, neophytadiene has been taken into consideration for perfumery applications and adds to the aroma profile of certain essential oils [23].

TABLE 1: PHYTOCHEMICALS IDENTIFIED IN THE PETROLEUM ETHER EXTRACT OF THE AERIAL PART OF *GRANGEA MADERASPATANA* BY GC-MS

Peak	R. Time	Area	Area%	Name
1	12.967	789503	0.31	1-Nonadecene
2	13.029	1722800	0.67	Nonadecane
3	13.426	12989905	5.08	Neophytadiene
4	13.883	683117	0.27	Oxirane,hexadecyl-
5	14.630	3095863	1.21	m-Camphorene
6	15.000	18037495	7.05	n-Hexadecanoicacid
7	15.074	2539588	0.99	Octadecane
8	15.590	1830278	0.72	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol
9	15.851	13985032	5.46	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol
10	16.200	6806678	2.66	Phytol
11	16.478	687158	0.27	2-Piperidinone,N-[4-bromo-n-butyl]-
12	16.615	4416675	1.73	9,12-Octadecadienoicacid(Z,Z)-
13	16.683	9834299	3.84	9,12,15-Octadecatrienoicacid,(Z,Z,Z)-
14	16.890	558641	0.22	Behenicalcohol
15	16.931	759564	0.30	Heneicosane
16	17.078	741208	0.29	Phytol,acetate
17	18.643	314173	0.12	Cyclohexane,[6-cyclopentyl-3-(3-cyclopentylpropyl)hexyl]-
18	18.690	440138	0.17	Heneicosane
19	19.738	667636	0.26	Heneicosane
20	20.656	867481	0.34	Cyclononasiloxane,octadecamethyl-
21	21.024	1802888	0.70	Docosane
22	22.642	2853309	1.11	Heneicosane
23	24.712	2850822	1.11	Tetracontane
24	24.994	6225866	2.43	Squalene
25	26.568	13112652	5.12	Tetracontane
26	26.965	2479093	0.97	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-
27	27.142	3208901	1.25	Glyceroltricaprylate
28	27.489	2899285	1.13	Octacosane,1-iodo-
29	27.844	7792666	3.05	Tetracontane
30	28.702	2062777	0.81	Octacosane,1-iodo-
31	29.313	59567617	23.28	Tetracontane
32	29.828	6894567	2.69	GlycerolTricaprylate
33	30.502	6276788	2.45	Triacontane,1-iodo-
34	30.990	7325163	2.86	Tetracontane
35	32.204	859537	0.34	Triacontane,1-iodo-
36	33.054	20325831	7.94	Tetracontane
37	33.335	4774344	1.87	Chondrillasterol
38	33.552	2839604	1.11	3-(Octanoyloxy)propane-1,2-diylbis(decanoate)
39	34.623	2191957	0.86	2H,6H-benzo[1,2-B:5,4-B']dipyran-6-one,5-hydroxy-7-(p-methoxyphenyl)-2,2-dimethyl-10-(3-methyl-2-but enyl)-
40	35.346	2211393	0.86	alpha-Amyrin
41	35.539	1115054	0.44	Tetracontane
42	38.650	3578213	1.40	Tetracontane
43	39.748	1210868	0.47	Hexacosyltrifluoroacetate
44	40.394	3959110	1.55	Phytyltetradecanoate
45	40.933	5718134	2.23	3,5,7-tris(trimethylsiloxy)-2-[3,4-di(trimethylsiloxy)phenyl]-4H-1-benzopyran-4-one

TABLE 2: MAJOR PHYTOCHEMICALS IDENTIFIED IN PETROLEUM-ETHER EXTRACT OF *GRANGEA MADERASPATANA* AERIAL PART

S. no.	Phytochemical Compound	RT (min)	Molecular Formula	Molecular weight	MS Fragment ions	Area (%)
1	Tetracontane	24.712, 26.568, 27.844, 29.313, 30.990, 33.054, 35.539, 38.650	$C_{40}H_{82}$	562	27, 41, 43, 57, 71, 85, 99, 113, 127, 141, 159, 169, 183, 197, 211, 225, 239	45.20
2	n-Hexadecanoicacid	15.000	$C_{16}H_{32}O_2$	256	40, 41, 43, 60,73, 85, 98, 115, 129, 143, 157, 171, 185, 199, 213, 227, 239, 256	7.05
3	(S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol	15.590 15.851	$C_{17}H_{24}O$	244	27, 41, 55, 67, 77, 91, 103, 115, 129, 141, 159, 173, 187, 201, 217, 229, 243	6.18
4	Neophytadiene	13.426	$C_{20}H_{38}$	278	27, 41, 55, 68, 82, 95, 109, 123, 137, 151, 179, 193	5.08

Characterization Data: Compound I as Tetracontane: Colorless amorphous powder, ^{1}H NMR (δppm, $CDCl_3$, 300 MHz) δ: 2.26 (2H, m, CH_2), 2.04 (2H, m, CH_2), 1.54 (4H, m, 2 X CH_2), 1.25 (68H, brs, 34 X CH_2), 0.87 (3H, t, J = 6.8 Hz, CH_3), 0.84 (3H, t, J = 6.3 Hz, CH_3). ^{13}C NMR (δppm, $CDCl_3$, 75 MHz) δ: 31.90 (CH_2), 29.67 (31 X CH_2), 29.63 (CH_2), 28.59 (CH_2), 27.36 (CH_2), 26.15 (CH_2), 25.89 (CH_2), 22.67 (CH_2), 14.09 (-CH_3).

Compound II as n-Hexadecanoicacid: Obtained white waxy compound. ^{1}H NMR (δppm, $CDCl_3$, 300 MHz) δ: 0.854 (3H, t, -CH_3), 1.260 (24H, m, 12 X –CH_2), 1.571 (2H, m, -CH_2-CH_2-COOH), 2.169 (2H, t, -CH_2-CH_2-COOH), 5.363 (1H, s, -CH_2-CH_2-COOH). ^{13}C NMR (δppm, $CDCl_3$, 75 MHz) δ: 177.13 (-COOH), 14.02 (-CH_3), 22.94 (CH_3-CH_2-), 34.35 (-CH_2-CH_2-COOH), 24.81 (-CH_2-CH_2-COOH), 28.96 (remaining –CH_2).

Compound III as (S,Z)-Heptadeca-1,9-dien-4,6-diyn-3-ol: Light brown powder,^{1}H NMR (δppm, $CDCl_3$, 300 MHz): δ 0.96 (3H, t, -CH_3), 1.33 (2H, m, -CH_2-CH_3), 5.48 (2H, m, -CH=CH-), 2.70 (2H, d, -CH_2), 5.89 (1H, t, CH_2-CH-), 5.23 (2H, d, CH_2-CH-), 1.29 (6H, m, -CH_2-), 1.42 (2H, m, -CH_2-), 2.01 (2H, m, -CH_2-). ^{13}C NMR (δppm, $CDCl_3$, 75 MHz): δ 115.10 (C-1), 65.15 (C-2), 75.80 (C-3), 70.65 (C-4), 64.40 (C-5), 73.25 (C-6), 19.60 (C-7), 123.80 (C-8), 133.10 (C-9), 32.47 (C-10), 30.76 (C-11), 30.98(C-12), 30.13 (C-13), 32.54 (C-14), 23.54 (C-15), 14.90 (C-16).

Compound IV as Neophytadiene: Grayish white oil, ^{1}H NMR (δppm, $CDCl_3$, 300 MHz): δ 1.01 (6H, d, 2 X -CH3), 1.06 (6H, d, 2 X -CH3), 1.83 (1H, m, -CH-), 1.65 (2H, m, -CH-), 1.25-1.29 (16H, m, -CH2-), 5.16 (2H, d, CH2-CH-), 6.25 (1H, t, CH2-CH-), 4.80 (1H, s, -CH_a-). 4.80 (1H, s, -CH_b-). ^{13}C NMR (δppm, $CDCl_3$, 75 MHz): δ 116.3 (C-1), 136.9 (C-2), 154.2 (C-3), 38.2 (C-4), 25.4 (C-5), 37.7 (C-6), 32.1 (C-7), 37.2 (C-8), 25.3 (C-9), 37.9 (C-10), 32.0 (C-11), 25.0 (C-12), 39.7 (C-13), 28.5 (C-14), 22.3 (-CH_3).

CONCLUSION: Phytochemical analysis of *Grangea maderaspatana* has identified various bioactive compounds, including fatty acids, terpenes, phytols and other organic compounds, which contribute to its medicinal potential. In the petroleum ether extract of aerial part of the plant found tetracontane (45.20% area at different retention time), n-hexadecanoic acid (7.05%area), (S,Z)-heptadeca-1,9-dien-4,6-diyn-3-ol (6.18% area at different R. Time) and neophytadiene (5.08% area) as major compounds on GC-MS analysis. The presence of these compounds suggests potential applications in pharmaceuticals, cosmetics and other industries due to their bioactive properties.

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CONFLICT OF INTREST: Authors declare no conflict of interest.

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