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Research Article

ANALYSIS OF VOLATILE COMPOUNDS IN THE SAP OF *AZADIRACHTA INDICA* (NEEM) USING GAS CHROMATOGRAPHY MASS SPECTROMETRY

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ABSTRACT

Azadirachta indica (Neem) has a dynamic role in various problems associated with human health. Each part of the Neem tree has some medicinal property and is thus commercially utilizable. The plant parts such as leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. The aim of this study is to identify and characterize the vital bioactive compounds from the Neem sap by Gas chromatography and Mass spectroscopy (GC-MS). The GC-MS analysis of the Neem sap revealed the presence of 30 volatile compounds. Among the 30 compounds, the most predominant compounds are fatty acids like Hexadecanoic acid and Pentadecanoic acid. Hence, this current attempt forms a basis for the biological characterization and importance of the compounds which could be exploited for future development of drugs.

KEYWORDS: GC-MS analysis; Volatile compounds; *Azadirachta indica*.

INTRODUCTION

Azadirachta indica is a member of mahogany family, Meliaceae. The taxonomic classification of Neem is as follows: Kingdom: Plantae, Order: Rutales, Suborder: Rutinae, Family: Meliaceae, Subfamily: Melioideae, Tribe: Melieae, Genus: Azadirachta, Species: indica[1]. Neem tree is mainly cultivated in the Indian subcontinent. It is consumed as a vegetable in some parts of the Asian subcontinent but mostly used as traditional medicine for centuries to cure multiple human diseases. For example, Neem components have been shown to have antifungal, anthelmintic, antibacterial, antiviral, anti-diabetic, contraceptive, and sedative effects[2,3]. Additionally, its constituents are applied in alternative (Ayurveda, Unani, Homeopathy) and modern medicine, e.g., for the treatment of diverse infectious, metabolic, or cancer diseases[4,5]. Many compounds have been found in the exudates of the, *Azadirachta indica* plant that have been used medically by humans. Studies of extracts from all major parts of Neem plant including leaves, flowers, fruits, and seeds, have shown promising chemopreventive and therapeutic effects in pre-clinical research[6]. So, this is now being widely used in pharmaceutical and cosmetics industries. Many compounds have been isolated from the extracts of various parts of the Neem tree. Quercetin and ß-sitosterol, were the first polyphenolic flavonoids purified from the fresh leaves of neem and were known to have antibacterial and antifungal properties. Neem plays role as free radical scavenging properties due to rich source of antioxidant. Azadirachtin and Nimbolide showed concentration-dependent antiradical scavenging activity[7]. The extract of *A. indica* leaves showed significant anti-inflammatory activity in cotton pellet granuloma assay in rats[8]. The leaf extract has shown virucidal activity[9]. Neem leaves also have the wound healing activity in both excision and incision wound models[10]. *Azadirachta indica* fruit skin extract and isolated ingredient Azadiradione showed significant antinociceptive property[11]. The medicinal utilities have been described, especially for leaf, fruit and bark. But research on the plant part, sap has not been investigated yet. Hence, this study is undertaken to find out the volatile compounds present in the sap of *A.indica* by using Gas chromatography and Mass spectroscopy technique.

MATERIALS AND METHODS

COLLECTION OF NEEM SAP

The green fruits of Neem were collected from the trees of Sathyamangalam, Tamil Nadu. Then, the white fluid was taken out from the fruits and stored in the separate beaker.

GC-MS ANALYSIS

GC-MS analysis of the sap of *A.indica* was performed using Thermo GC- Trace ultra-version 5.0 and Thermo MS DSQ II. The equipment has a DB 35 – MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas

was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1µl was employed. The injector was operated at 250 °C and the oven temperature was programmed from 70 ˚C with an increase of 6 ˚C /min to 260 ˚C. Mass spectra were taken at 70 eV and the total GC-MS running time was 40.53 min. The peaks corresponding to the components were identified based on Willey and NIST libraries as well as comparison of their retention indices. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The results obtained have been tabulated.

DISCUSSION

The present study was undertaken to find out the volatile bioactive compounds present in the sap of *A.indica* by using Gas chromatography and Mass spectroscopy. In this study, the Neem fruits were collected and the white colored fluid was separated out. The volatile compounds present in it was characterized by using GC-MS.

GC-MS ANALYSIS

The Neem sap was analysed for the detection of bioactive compounds in it by using GC-MS. The GC-MS profile of the sap of *A.indica* is represented in figure 2 which showed the prominent peaks for the presence of 30 bioactive compounds. The probable volatile compounds corresponding to the peaks with their retention time (RT), molecular formula, molecular weight (MW), structure and concentration (peak area %) are tabulated in Table 1.

From the analysis, it is revealed that there are 30 probable volatile compounds present in the Neem sap. These constituents belong to 15 classes of compounds including hydrocarbons, fatty acids, esters, alcohols, isothiocyanate, thiocyanate, pyrazine, aromatics, alkamides, cyanides, steroids, halocompounds, urea and N-hydroxyimine derivatives, unsaturated alkenamides, alkyne and indol.

From the above characterized compounds, Hexadecanoic acid has anti-tumor activity to human leukemic cells as well as murine cells[12]. Hexadecanoic acid (palmitic acid) has been reported to induce NF-κB activation in HaCaT keratinocytes, which is an important pathway involved in cancer development. Besides, fatty acids exhibit cytotoxicity against HeLa cells, n-Hexadecanoic acid showed significant cytotoxicity against oral cancer (KB), breast cancer (MCF-7) and small cell lung cancer (NCI-H187)[13]. It also acts against inflammation[14]. Docosane owns antimicrobial activity against both gram positive and gram negative bacteria[15]. Docosenamide in its biosurfactant owes to its anticancerous and antiviral activities[16].

CONCLUSION

The presence of various volatile bioactive compounds in the Neem sap justifies the use of this in treating various ailments. Hence, this result also enhances the traditional usage of *A.indica* which possesses a number of bioactive compounds. Therefore, it is recommended as a source of pharmaceutical importance and can be used as target for many known and unknown ailments. Future work on this study is to isolate and characterize the useful bioactive compounds for treating various diseases.

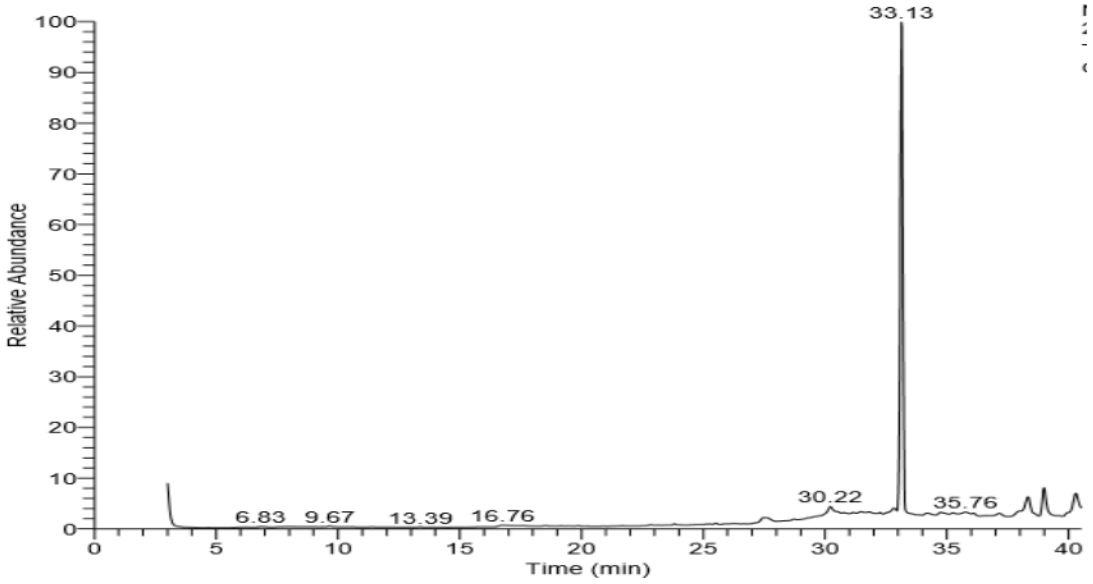
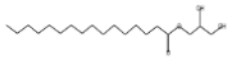
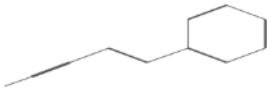
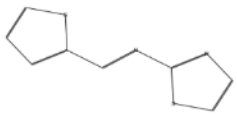
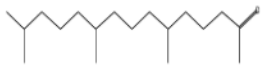
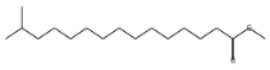
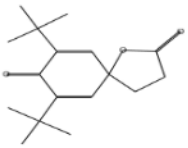
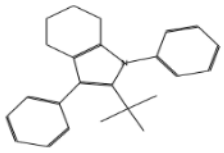
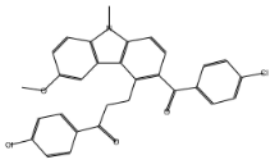
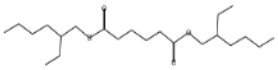
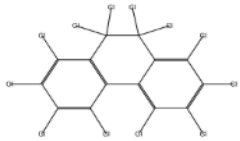
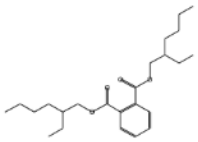
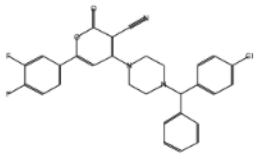
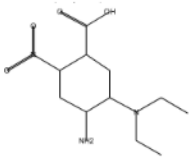
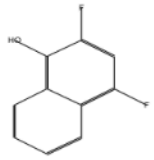
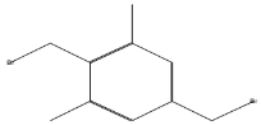
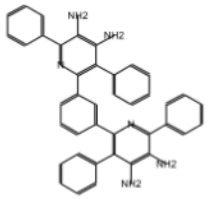
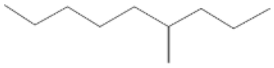
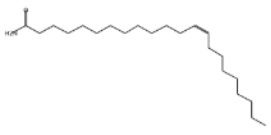
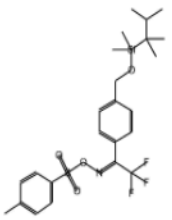


Figure 1: GC-MS Profile of *Azadirachta indica* sap

S.No	Retention Time	Compound name	Molecular Formula	Molecular weight	Area%	Structure
1	6.10	Hexadecanoic acid, 2,3-dihydroxypropyl ester	C19H38O4	330	0.13	
2	6.83	Docosane	C22H46	310	0.25	
3	7.90	1-Phenylpent-1-en-3-yne	C11H10	142	0.26	
4	9.67	Tetradecene	C14H28	196	0.25	
5	16.74	7-Methoxy-1-tetralone Ethylene Dithioketal	C13H16OS2	252	0.66	
6	18.47	2-(2-Thienylmethyleneamino)thiazole	C8H6N2S2	194	0.16	
7	19.85	2-Pentadecanone, 6,10,14-trimethyl-(CAS	C18H36O	268	0.17	
8	21.66	Pentadecanoic acid, 14-methyl-, methyl ester	C17H34O2	270	0.18	
9	22.84	3-Methoxy-9-phenylphenanthrene	C21H16O	284	0.23	
10	23.82	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	276	0.22	

11	25.51	2-tert-Butyl-1,3-diphenyl-4,5,6,7-tetrahydroindole	C24H27N	329	0.33	
12	26.26	3-(4-Chlorobenzoyl)-6-methoxy-9-N-methyl-4-[2'-(4-chloro benzoyl)ethyl]carbazole	C30H23Cl2N O3	515	0.24	
13	27.47	Docosane	C22H46	310	1.76	
14	28.73	Triacontane	C30H62	422	0.18	
15	30.22	Hexanedioic acid, bis(2-ethylhexyl) ester	C22H42O4	370	2.37	
16	31.15	Nonacosane	C29H60	408	0.18	
17	31.46	Dodecachloro-9,10-dihydrophenanthrene	C14Cl12	588	0.47	
18	31.81	[1Z,2(1R,2R)]-1-(Trimethylstannyl)-2-vinylcyclopentyl)ethen yl 2,2,4,4-tetramethyl-1,3-dioxazolidine-3-carboxylate	C20H35NO3 Sn	457	0.39	
19	32.26	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester (CAS)	C24H38O4	390	0.17	
20	33.13	(2RS)-1,3,8-trimethyl-4-propyl-5-ethyl-2-(1-hydroxyethyl)-7methoxycarbonyleth yl-6,.gmma.-methylenecarbonyl-porphine	C36H42N4O4	594	71.27	

21	34.17	6-(3,4-Difluorophenyl)-3-cyano-4-[N-[(4-chlorophenyl)(phenyl)methyl]piperazino]-2H-pyran-2-one	C29H22ClF2 N3O2	517	0.58	
22	34.74	4-Amino-3-diethylamino-6-nitrobenzoic acid	C11H15N3O4	253	0.71	
23	35.25	2,4-Difluoro-1-hydroxynaphthalene	C10H6F2O	180	0.22	
24	35.78	1,4-Bis(bromomethyl)-2,6-dimethylbenzene	C10H12Br2	290	0.83	
25	36.11	m-Bis(3,4-diamino-2,5-diphenyl-6-pyridino)benzene	C40H32N6	596	0.37	
26	37.17	2,9-bis(2',6'-dimethoxyphenyl)-1,10-phenanthroline	C28H24N2O4	452	0.76	
27	38.33	Nonane, 4-methylene	C10H20	140	6.95	
28	39	13-Docosenamide	C22H43NO	337	5.57	
29	39.92	1-[4-[[[(1,1,2-Trimethylpropyl)dimethylsilyl]oxy]methyl]phe nyl]-2,2,2-trifluoro-1-ethanone O-tosyl oxime	C24H32F3NO 4SSi	515	0.13	

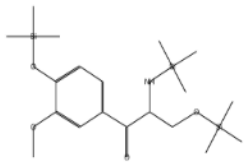
30	40.28	1-Trimethylsilyloxy-2-trimethylsilylamino-3-(3'-methoxy-4'-trimethylsilyloxy-phenyl) propanone	C19H37NO4S i3	427	3.99	

Table 1: Volatile components identified in the sap of *Azadirachta indica*

ABBREVIATIONS

GC-MS - Gas Chromatography Mass Spectrometry
RT - Retention Time
MW - Molecular Weight
NIST - National Institute of Standards and Technology

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