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Research Article

GC-MS analysis of naturally occurring gum exudates of *Azadirachta indica A. Juss.*

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Abstract

The present investigation was carried out to analyze the active constituents present in the gum exudates of *Azadirachta indica A. Juss.* Meliaceae. Twelve compounds were identified by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The predominant compounds were fatty acids like Hexadecanoic acid, oleic acid ethyl ester, stearic acid ethyl ester, linoleic acid ethyl ester, methyl ricinoleate and ricinoleic acid.

Keywords: *Azadirachta indica* gum, GC-MS analysis, Fatty acids

1. Introduction

The gum exudate from the stem of *Azadirachta indica* tree (Neem tree) is a mixture of proteins and polysugars. The proteins are linked very tightly to the polysaccharides, which constitute the major components. Due to this complexity, the structural elucidation of proteins and polysaccharides from neem gum is a multifarious one [1]. Presence of D- glucose, D-glucoronic acid, L-arabinose, L-fucose, mannose, xylose, rhamnose, D- glucosamine, aldobiuronic acid, serine, threonine and aspartic acid in *Azadirachta indica* gum were reported by few authors [2,3]. In addition to that it also found to contain organic fatty acids [4]. *Azadirachta indica* gum is widely used in various industries for its commercial applications. It is used in cosmetic (facial masks, lotions, face powder), paper (adhesive and strengthening the paper), pharamaceutical (antiseptic creams, tablet binder, and coater), textile (dyeing and printing of fabrics) and food industry (stabilizing agent, gels and thickening agent). Researchers have employed a wide variety of analytical techniques for the characterization of polysaccharides from gum, probably the most widespread over recent years being gas chromatography- mass spectroscopy (GC-MS). However, perusal of literature reveals that GC-MS analysis of *Azadirachta indica* gum is fully focussing on the isolation and characterization of polysaccharides (after derivatization) and hence the present investigation was undertaken to identify the various constituents without any derivatization prior to GC-MS analysis.

2. Materials and Methods

2.1 Collection and purification of *Azadirachta indica* gum

The gum exudates of *Azadirachta indica A. Juss. Meliaceae* was collected from PSG College of Arts and Science campus (Coimbatore, Tamil Nadu, India) and identified taxonomically and authenticated by the Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India. The gum samples were refrigerated under -5^{o} C until use. The collected gum samples were washed with double distilled water to remove impurities like bark, dust and sand etc. Estimated quantity of gum was put into 50 ml of double distilled water and kept aside for overnight. Subsequently next day, after the complete dissolution of gum, it was filtered through whatman filter paper. Then it was kept in a desiccator for five days to obtain glassy mass of purified gum sample.

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2.2 GC- MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1µM df, composed of 100% Dimethyl poly diloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1 mL/min and an injection volume of 0.5 µL was employed (split ratio of 10:1) injector temperature 250ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9 min isothermal at 280 ºC. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. The total GC running time was 42 min.

3. Results and discussion

The physiochemical parameters like pH, colour, odour, taste and solubility of *Azadirachta indica* gum (AI gum) were tested and given in Table 1. From the results it can be seen that the AI gum is acidic, yellowish brown in colour, odourless and non- bitter in taste. The results of solubility clearly shows that AI gum completely soluble in water. However the gum is sparingly soluble in acetone and ethanol.

Table 1: Physiochemical properties of AI gum

Properties	
pH	Acidic
Colour	Yellowish- brown
Odour	-
Taste	Non- bitter
Solubility	
(a) Cold water	Completely soluble
(b) Warm water	Completely soluble
(c) Acetone	Sparingly soluble
(d) Ethanol	Sparingly soluble

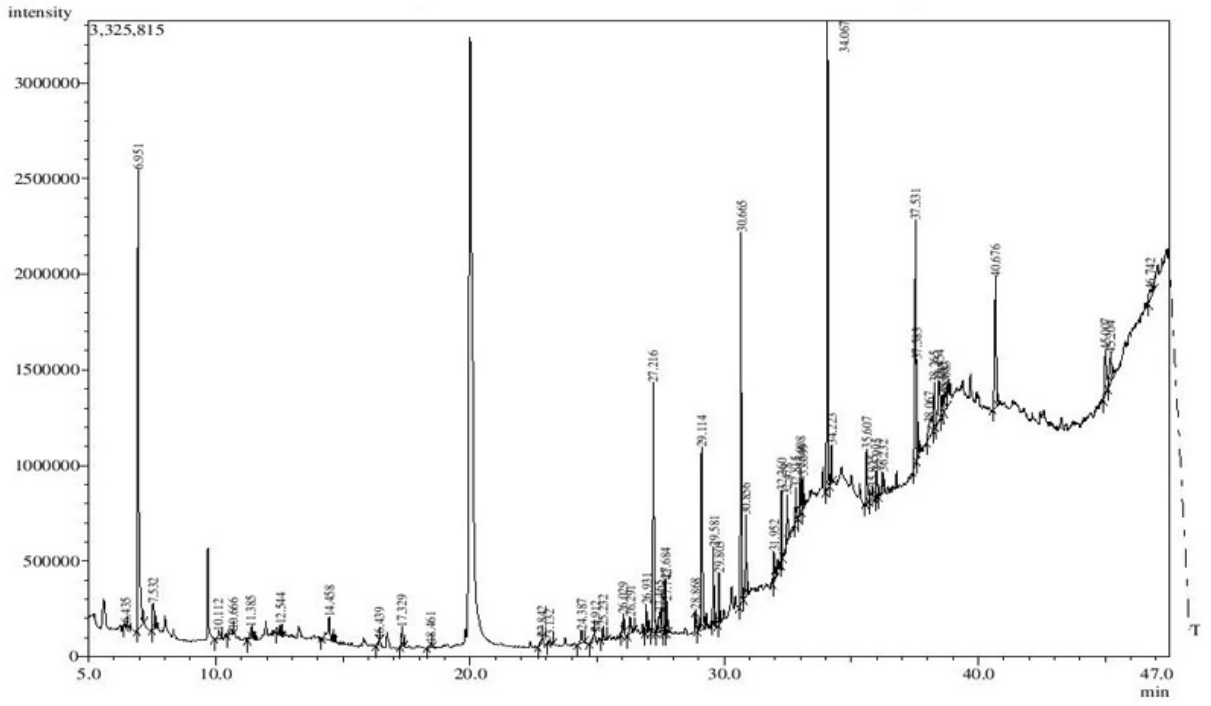


Figure 1: GC-MS spectrum of gum exudates of *Azadirachta indica*

Figure 1 represents the GC-MS spectrum of AI gum. It revealed the presence of several components and identified through comparison of the fragmentation patterns in the resulting mass spectra using NIST and WILEY mass spectral data base of the gas chromatograph system. Values of retention time, mass peak, molecular formula,

molecular weight and compound names are presented in Table 2. The structures of identified compounds were given in Figure 2.

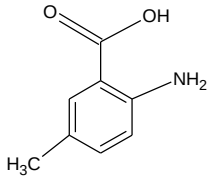
Table 2: Characteristics of suggested compounds identified from GC-MS of AI gum

Peak scan	Compound name	Molecular formula	Molecular weight	Retention time	Fragmentation peaks
245	2-Amino-5-methylbenzoic acid	$C_8H_9NO_2$	151	7.033	26, 51, 66, 77, 89, 104, 133, 151
2141	3,7-dimethyl-1,7-octadien-3-amine	$C_{10}H_{19}N$	153	22.83	30, 41, 69, 70, 93, 109, 121, 138
2667	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol	$C_{15}H_{24}O_2$	236	27.21	27, 41, 55, 67, 79, 91, 107, 121, 133, 149, 163, 175, 192, 218, 236
2895	Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester	$C_{16}H_{26}O_2$	250	29.114	27,41,43, 69, 81, 95, 110, 123, 137, 150, 166, 175, 190, 208
2951	Hexadecanoic acid	$C_{18}H_{36}O_2$	284	29.58	39, 41, 57, 70, 88, 101, 115, 129, 143, 157, 171, 185, 199, 213, 227, 241, 255, 284
3081	13-Hexyloxacyclotridec-10-en-2-one	$C_{18}H_{32}O_2$	280	30.66	27, 41, 55, 67, 81,96, 98, 123, 137, 151, 166, 280
3235	Oleic acid, ethyl ester	$C_{20}H_{38}O_2$	310	31.95	27, 41, 55, 69, 83, 88, 101, 123, 137, 152, 180, 222, 264, 266
3272	Stearic acid, ethyl ester	$C_{20}H_{40}O_2$	312	32.25	39, 41, 57, 70, 88, 101, 115, 129, 143, 157, 171,1 99, 213, 227, 269, 312
3298	Linoleic acid, methyl ester	$C_{19}H_{34}O_2$	294	32.475	41, 55, 67, 81, 95, 109, 123, 136, 150, 164, 263, 294
3489	1,4 Dioxaspiro[4.5]decane	$C_8H_{14}O_2$	142	34.06	27, 41, 55, 67, 81, 86, 99, 113, 142
3911	Ricinoleic acid methyl ester	$C_{19}H_{36}O_3$	312	37.58	27, 41, 55, 69, 74, 84, 98, 124, 137, 148, 166, 198, 294
4282	Ricinoleic acid	$C_{18}H_{34}O_3$	298	40.67	43, 55, 69, 83, 97, 124, 137, 148, 166, 184

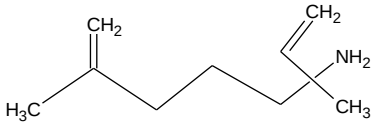
Compound 1 was identified as 2-Amino-5-methylbenzoic acid with molecular weight of 151 g mol^{-1} separated under retention time of 7.033 min. The compound is characterized by 8 fragmentation peaks and mass peak value of 74. The second compound separated was 3,7-dimethyl-1,7-octadien-3-amine with base peak at 70 and mass peak value of 23 with retention time of 22.83 min. 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol was separated as compound 3 with base peak at 193 and mass peak at 134. Compound 4 was idendified as Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester at 29.114 min with base peak at 153 (mol wt. 250 g mol^{-1}). The compound 6 was fatty acid named ethyl hexadecanoate under the retention time of 29.58 min with base peak at 88.1 and mass peak at 85. 13-Hexyloxacyclotridec-10-en-2-one was separated under the retention time of 30.66 min as compound 7. Hexadecanoic acid was separated as compound 8 with molecular weight of 310 g mol^{-1} with base peak and mass peak at 55, 119 respectively. Linoleic acid ethyl ester (R_t time. 32.48 min) was identified as compound 9 has a base peak at 67 and mass peak at 104. 1,4 Dioxaspiro[4.5]decane was identified as compound 10 with base peak at 99 and mass peak at 107. Consequently under a retention time of 37.58 min compound 11 was identified as methyl ricinoleate with base and mass peak at 98 and 130 respectively. The compound 12 was named as Ricinoleic acid with molecular weight of 298 g mol^{-1} has a base peak at 99 and mass peak at 199 under the retention time of 40.67 min.

Among the identified 12 compounds, fatty acids constitute a major percentage (50 %). Ethyl hexadecanoate is recommended to be antioxidant, hypocholesterolemic, nematicide, pesticide [5] and sesquesters spleen function and shows anti- inflammatory effect [6]. Oleic acid ethyl ester is suggested to be plasticizer and

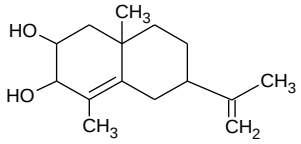
lubricant in pharmaceutical industries. Stearic acid ethyl ester can be used as anti-viral and anti- inflammatory drugs and linoleic acid ethyl ester helps to prevent cardiovascular diseases [7]. Methyl rincinoleate and ricinoleic acid exerts analgesic and anti-inflammatory effects [8].



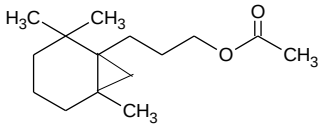
2-Amino-5-methylbenzoic acid



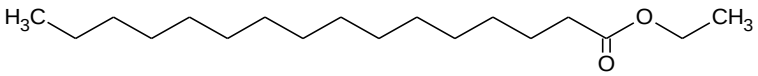
3,7-Dimethyl-1,7-octadien-3-amine



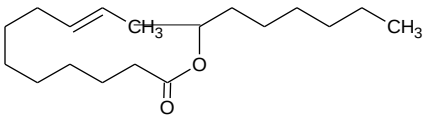
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalene-2,3-diol



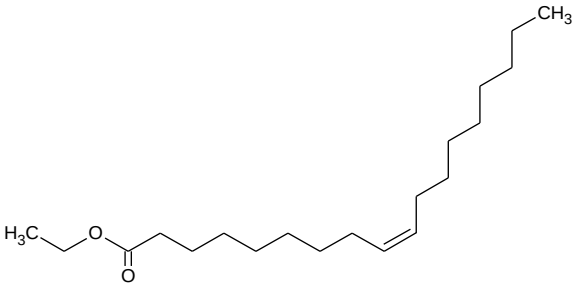
Acetic acid, 1-[2-(2,2,6-trimethyl-bicyclo[4.1.0]hept-1-yl)-ethyl]-vinyl ester



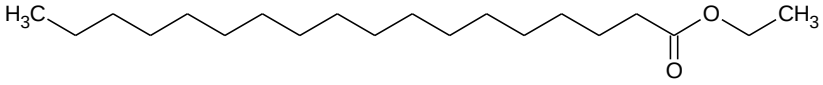
Hexadecanoic acid



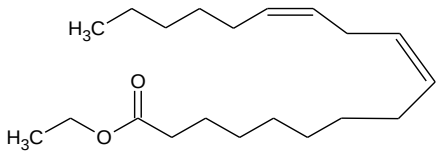
13-Hexyloxacyclotridec-10-en-2-one



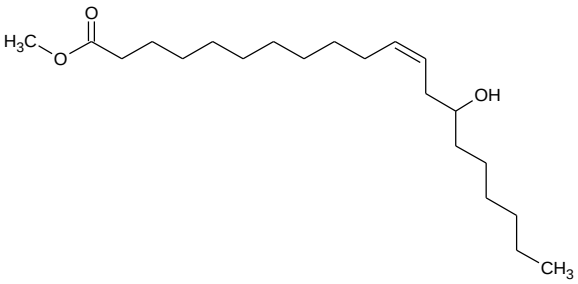
Oleic acid ethyl ester



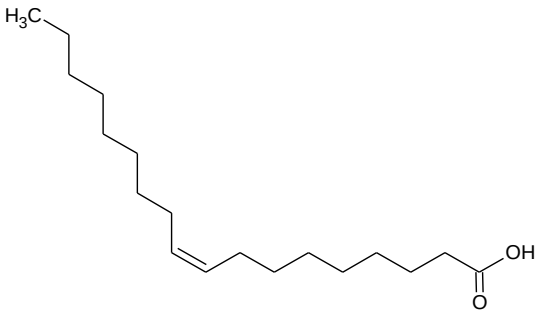
Stearic acid ethyl ester



Linoleic acid ethyl ester



Ricinoleic acid methyl ester



Ricinoleic acid

Figure 2: Structures of identified compounds from *Azadirachta indica* gum

4. Conclusion

The above identified compounds in *Azadirachta indica* gum were not reported so far. GCMS analysis is the first step towards understanding the nature of active principles in the plant extracts and will be helpful for further detailed study. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results.

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