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Research Article

CHEMICAL COMPOSITION OF METHANOL EXTRACT OF THE LEAVES OF *MELIA AZEDARACH* L.

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ABSTRACT

The aim of the study was to investigate the presence of phytochemical compounds from the leaves of *Melia azedarach* L., using methanolic extraction. The phytochemical compound screened by GC-MS method. 48 bioactive phytochemical compounds were identified in the methanolic extract of *M. azedarach*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. A large variety of compounds have been detected in *M. azedarach* including Flavonoid, phytosterols, Diterpene, alkane hydrocabon, n-alkanoic acid , vitamin-E and Tri-terpene, Terpene alcohol.

Keywords: *Melia azedarach* L., GC-MS method, phytochemical compounds.

INTRODUCTION

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Natural products have been a source of drugs for centuries[1]. In the last few years gas-chromatography mass-spectrometry has become firmly established as a key technological platform for metabolite profiling in plant[2,3,4,5,6]. GCMS based metabolome analysis has profound applications in discovering the mode of action of drugs or herbicides and helps unravel the effect of altered gene expression on metabolism and organism performance in biotechnological applications.

M. azedarach is traditionally been used as anthelmintic, antilithic diuretic, emmenagouge, astringent and stomachic. Various scientific studies reported the analgesic, anticancer, antiviral, antimalarial, antibacterial, antifeedent and antifertility avtivity of this plant[7,8].

Thus, as the experimental plant species possess immense medicinal properties, therefore the aim of the present study is to identify the biochemical compounds of *M. azedarach* by using the methanolic leaf extract through Gas chromatography – Mass Spectrum analysis.

MATERIAL AND METHOD

(i) Preparation of plant material

The fresh leaves samples of plant *M. azedarach* was collected the campus of University of Rajasthan, Jaipur and washed individually under running tap water to remove any traces of soil particles and other dirt. The sample were dried at 60°C for 2 days in an oven. They were then macerated to powder form with a mixer grinder.

(ii) Preparation of sample for GC/MS study

About 20 grams of the plant sample powdered were soaked in 100 ml methanol individually. It was left for 24 hours so that alkaloids, flavonoids and other constituents if present will get dissolved. The methanol extract was filtered using Whatman No.1 filter paper and the residue was removed. It was again filtered through sodium sulphate in order to remove the traces of moisture.

(iv) Gas chromatography – Mass Spectrum analysis

The GC-MS analysis of the plant extract was made in a (QP 2010 Plus SHIMADZU) instrument under computer control at 70 eV. About 1µL of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 minutes. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT).While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The X-axis showed the RT and the Y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass .The M/Z (Mass / Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule.

Before analyzing the extract using Gas Chromatography and Mass Spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane).

Identification of components

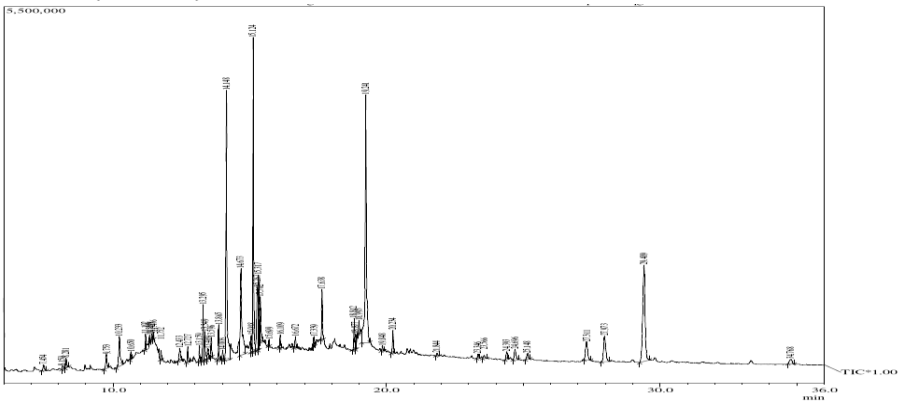
The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. NIST08.LIB[9], WILEY8.LIB[10] library sources were used for matching the identified components from the plant material.

RESULTS AND OBSERVATION

Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in methanolic leaf extract of *M. azedarach*, shown in Table-1 In the GC-MS analysis, 48 bioactive phytochemical compounds were identified in the methanolic extract of *M. azedarach*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight and molecular formula. In the present investigation a variety of compounds have been detected in p *M. azedarac,h* including Kampherol, Quercetin (Flavonoid), stigmasterol, β-sitosterol, Campesterol (phytosterols), Phytol (Diterpene), 3-Methyldecane, Heptadecane (alkane hydrocabon), hexadecanoic acid, Pentadecanoic acid (n-alkanoic acids) , Beta- Carotene, tocopherol (vitamin-E) and squalene, 1-Eicosanol (tri-terpene), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Terpene alcohol).

The highest peak area (%) of 16.47 was obtained by Quercetin (flavonoid) with retention-time 19.241 and the lowest peak area

(%) of 0.17 was obtained by 2,8-Dimethyl-2-(4,8,12-Trimethyltridecyl)-6-Chromanol (Vitamin E) with retention-time 21.844 (Table-1; Graph 1).



Graph 1: GCMS Spectra of methanolic extract of *Melia azedarach* L.

Table1: Phytocomponents identified in the methanolic leaf Extract of Melia *azedarach* by GC-MS

S.No	R.T	Area	Name of the compound	Peak Area %	M.F	M.Wt	Compound Nature
1	7.454	335309	2,3-Dihydro-3,5-Dihyroxy-6-Methyl-4H-Pyran-4-One	0.43	$C_6H_8O_4$	144	Aromatic flavoring agent
2	8.15	172083	2,3-Dihydrobenzofuran	0.22	C_8H_8O	120	Essential oil
3	8.281	410044	5-Hydroxypipecolic acid	0.52	$C_6H_{11}NO_3$	145	Imino acid
4	9.753	749285	Ethoxytriethylsilane	0.95	$C_8H_{20}OSi$	160	dibasic esters
5	10.233	1768374	4-Methyl-2-hexanone	2.25	$C_7H_{14}O$	114	Aromatic flavoring agent
6	10.65	179941	Pentadecane	0.23	$C_{15}H_{32}$	212	n-alkanes
7	11.192	536701	Limonene	0.68	$C_{10}H_{16}O$	152	monoterpenes
8	11.335	205410	Pyrazol-5(2H)-one	0.26	$C_{11}H_{14}N_2O_3$	222	Flavonoids
9	11.423	144433	1-Hexadecene	0.18	$C_{16}H_{32}$	224	alpha-olefins
10	11.476	158570	Nonadecane	0.2	$C_{19}H_{40}$	268	Saturated aliphatic hydrocarbon
11	11.752	206390	Caryophyllene oxide	0.26	$C_{15}H_{24}O$	220	flavor and fragrance agents
12	12.433	694146	1-Acetyl-4-hydroxy-pyrrolidin-2-one	0.88	$C_6H_9NO_3$	143	pyrrolidones
13	12.727	462945	n-Tetradecanoic acid	0.59	$C_{14}H_{28}O_2$	228	Saturated fatty acid
14	13.159	511509	1,2-Dihexylcyclopropene-3-carboxylic acid	0.65	$C_{16}H_{28}O_2$	252	n-alkanoic acids
15	13.295	1763004	9-Eicosyne	2.24	$C_{20}H_{38}$	278	Saturated aliphatic hydrocarbon
16	13.349	1011129	6,10,14-Trimethyl-2-pentadecanone	1.28	$C_{18}H_{36}O$	268	essential oil
17	13.469	598992	E-6-Octadecen-1-ol acetate	0.76	$C_{20}H_{38}O_2$	310	Fatty acids
18	13.596	549025	Citronellyl propionate	0.7	$C_{13}H_{24}O_2$	212	flavor and fragrance agents
19	13.865	961166	Hexadecanoic acid, methyl ester	1.22	$C_{17}H_{34}O_2$	270	methyl ester
20	14.003	395376	Carvacrol	0.5	$C_{10}H_{14}O$	150	monoterpenoid phenol
21	14.148	12189399	Palmitic acid	15.49	$C_{16}H_{32}O_2$	256	Saturated fatty acid
22	14.673	5439416	1,5-Anhydro-2-deoxyhex-1-enitol	6.91	$C_6H_{10}O_4$	146	unsaturated sugars
23	15.048	495739	Linolenic acid, methyl ester	0.63	$C_{19}H_{32}O_2$	292	poly unsaturated fatty acid
24	15.124	8690970	Phytol	11.04	$C_{20}H_{40}O$	296	Diterpene
25	15.267	2797730	Methyl linoleate	3.56	$C_{19}H_{34}O_2$	294	polyunsaturated fatty acid
26	15.317	2697246	9,12,15-Octadecatrienoic acid	3.43	$C_{19}H_{32}O_2$	292	n-alkanoic acids
27	15.382	1550682	Stearic acid	1.97	$C_{18}H_{36}O_2$	284	n-alkanoic acids
28	15.699	163832	2,6,10-Trimethyl,14-Ethylene-14-Pentadecne	0.21	$C_{20}H_{38}$	278	olefins
29	16.109	370653	2-Propenoic acid, 2-methyl-, 2-(dimethylamino)ethyl ester	0.47	$C_8H_{15}NO_2$	157	ethyl ester
30	16.672	428859	4,8,12,16-Tetramethylheptadecan-4-olide	0.54	$C_{21}H_{40}O_2$	324	isoprenoid
31	17.339	202988	1,3,5-Trisilacyclohexane	0.26	$C_3H_{12}Si_3$	132	Saturated hydrocarbon
32	17.638	1593008	Palmitic acid	2.02	$C_{19}H_{38}O_4$	330	monoglyceride
33	18.812	1074988	Tetracosanoic acid, 3-oxo-methyl ester	1.37	$C_{25}H_{48}O_3$	396	methyl ester
34	18.877	486765	Hexacosane	0.62	$C_{26}H_{54}$	366	unsaponifiable matter
35	18.995	428607	Stearic acid chloride	0.54	$C_{18}H_{35}Cl_O$	302	Unsaturated fatty acid
36	19.241	12959406	Quercetin	16.47	$C_{15}H_{10}O_7$		Flavanoids
37	19.848	218677	13-Docosenamide	0.28	$C_{22}H_{43}NO$	337	Alkyl amides
38	20.234	754714	Squalene	0.96	$C_{30}H_{50}$	410	Triterpene
39	21.844	136222	2,8-Dimethyl-2-(4,8,12-Trimethyltridecyl)-6-Chromanol	0.17	$C_{27}H_{46}O_2$	402	Vitamin E
40	23.346	203870	Gamma- Tocopherol	0.26	$C_{28}H_{48}O_2$	416	Vitamin E
41	23.566	163174	1-Eicosanol	0.21	$C_{20}H_{42}O$	298	Triterpene

42	24.393	390376	Beta- Carotene	0.5	$C_{40}H_{56}$	536	Vitamin E
43	24.696	685915	alpha-Tocopherol-beta-D-mannoside	0.87	$C_{35}H_{60}O_7$	592	Glucoside
44	25.148	350181	Kamphferol	0.44	$C_{15}H_{10}O_6$	286	Flavanoids
45	27.311	1454310	Beta-Sitosterol	1.85	$C_{29}H_{50}O$	414	Phytosterol
46	27.973	2031508	Stigmasterol	2.58	$C_{29}H_{48}O$	412	Phytosterol
47	29.409	8509681	Campesterol	10.81	$C_{28}H_{48}O$	400	Phytosterol
48	34.768	414859	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.53	$C_{20}H_{40}O$	298	T e r p e n e alcohol
		78697607		**100**			

DISCUSSION

Medicinal plants are used in traditional treatments to cure variety of diseases. In the last few decades there has been an exponential growth in the field of herbal medicine. Thus, for the above mentioned reason and bearing in mind its medicinal importance, the plant species *M. azedarach* were selected to analyze by GC-MS technique and to explore the major and minor phytoconstituents present in the respective plant species.

The preliminary phytochemical screening shows the presence of saturated and unsaturated fatty acids, hydrocarbons, glycosides, polysaccharides, phenolic compounds, alkaloids, flavonoids, phytosterols, monoterpenes, triterpenes, sesquiterpenes, essential oil and vitamins.

The Methanolic extract of the plant showed 48 constituents, the major constituents weing Phytol (11.04%- Diterpene), Quercetin (16.47%- Flavanoids), Palmitic acid (15.49%- Saturated fatty acid). 9,12,15-Octadecatrienoic acid (3.43%- n-alkanoic acids) serves as an anti inflammatory, hypocholesterolemic cancer preventive,

hepatoprotective, nematicide, insectifuge, antihistaminic properties[11]. Some minor components like 2,3-Dihydro-3,5-Dihyroxy-6-Methyl-4H-Pyran-4-One (0.31% - flavoring agent) and 4-Methyl-2-hexanone (2.25%- flavoring agent) works as a novel potent aroma compound in a dairy product[12,13]. While, 2,3-Dihydrobenzofuran (0.22%) is an essential oil used in the treatment of diabetic retinopathy and arthritis[14]. 5-Hydroxypipecolic acid (0.52 %- imino acid) showed platelet aggregation inhibition and larvicidal activity[15]. Pentadecane (0.23%- n-alkanes) was used as pesticide[16]. Limonene (0.68%- Monoterpenes) prevents dehydration, the inhibition of microbial, act as natural food flavourings, fragrances and aromatherapy[17]. Pyrazol-5(2H)-one (0.26%- Flavonoids) on the other hand possessed a wide spectrum of biological activities, such as antiinflammatory, antipyretic, analgesic[18]. The most common role of vitamin E is its antioxidant effect, protecting molecules and tissues from deleterious free radicals. Biosynthesis of various biologically active steroids necessary for the normal vital activity of plants starts from phyotsterols, including stigmasterol (2.58%)[19]

The present work and detection of compounds is in consonance with the work reported by other scientists in different plant species viz., *Minuartia meyeri* [20], *Mentha spicata* and *Camellia sinensis*[21], *Kalanchoe pinnata* [22].

CONCLUSION

Thus the GC-MS analysis of methanolic extract of *M. azedarach* showed a highly complex profile, containing approximately 48 components, mainly quassin, squalene, stigmasterol, vitamin E and flavonoids. This study may be useful to explore the pharmacological and biosynthetic activity of the plants further.

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