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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2020, Vol. 36, No.(6):
Pg. 1229-1234

Separation and Identification of New Phytocomponents in Methanolic Extract of Leaves of Hairknot Plant (*Pergularia daemia*) by GC-MS Analysis

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http://dx.doi.org/10.13005/ojc/360631

(Received: September 30, 2020; Accepted: November 01, 2020)

ABSTRACT

The investigation was designed to trigger the complete chemical constituents present in the methanolic extract of leaves of hair knot plant; botanically known as *Pergularia daemia* belongs to the family of the *Asclepiadaceae* one of the endemic plants of Andhra Pradesh, India by Agilent Technologies Gas Chromatography–Mass Spectrometry and components were well separated on HP-5 MS capillary column consisting of stationary phase of 5% phenyl 95% methylpolysiloxane. The resulted compounds were appropriate to the NIST (National Institute of Standards and Technology) library and the study indicated the presence of different phytochemical compounds. A total of 51 compounds were recognized in the methanolic extract of *Pergularia daemia* leaves. Further, five new compounds were separated and four of biological importance viz. Pentadecane, Tetratriacontane, Dibutyl phthalate and Squalene were identified. The results of the importance of biologically active phytochemicals in the study recommend, *P. daemiaas* as a plant of phytopharmaceutical reputation.

Keywords: Bioactive compounds, Phytochemicals, GC-MS, Pergularia daemia, Asclepiadaceae.

INTRODUCTION

The use of plants in several countries as a major source of medicines is hereditary and has an important element of the health care system. India has a lengthy history and strong base for the traditional herbal medicinal system and is suitably called as the botanical garden of the world[1]. Biological studies are used to extract wide medicinal properties from plants[2].

As synthetic drugs which are consumed by human beings may have various side effects and may lead to serious health complications. So, herbal medicine was the life saving drug with fewer side effects and minimum cost effort. Generally,

the progress of herbal medicine was carried by the preliminary screening of the constituents in plant sample extracts. The plant chemicals that are not necessary for growth and development but have the properties to protect or prevent diseases are called phytochemicals. The plant produces these chemicals to protect them and they have the ability to treat human diseases also[3]. There are many phytochemicals, which have their own pharmacological importance[4]. The plant *Pergularia daemia* is known as "Dushtupatige" in Telugu and "Uttaravaruni" in Sanskrit. *Pergularia daemia* dried leaf is effectively used in treating asthma, dysmenorrheal, rheumatic fever, bronchitis, amenorrhea, and wounds, etc.[5]. It has pharmacological importance like anti-microbial, anti-fungal properties, anti-rheumatic, anti-arthritic, anti-inflammatory, anti-proliferative, and a good anti-oxidant[6-9]. The previous analysis only reported for potent snack venom potency of four constituents ß-sitosterol, ß-amyrin, alpha-amyrin, and Lupeol from leaves of *Pergularia daemia* by GC-MS method[10]. The study on ethanolic extract of leaves reports the existence of methyl Ester pentadecoinic acid, 14-methyl-methyl Esterethyl 9-12-15-Octodecotrionate, hexadecanoic acid, and 4-4 chlorobenzyl-1-cyclohexoxyl l-5-tosylamino-1, H-123 and also ß-sitosterol, lupeol, lupeol acetate, alpha-beta amyrin and its acetate in entire plant and flower. While lupeol-3 Betatranscrotonate and oleanolic acid acetate in dried entire plant[11]. These reports are helpful for our investigation to go for a complete analysis.

The medicinal assets of *P.daemia* may be due to the occurrence of its phytochemical ingredients which is not yet explored thoroughly in the Andhra Pradesh region of India except other species in the family[12]. Moreover, the preliminary studies[13,14] on the phytochemicals of *P. daemia* also encouraged for the present study. In the present investigation, the Gas Chromatographic-Mass Spectrometric Analysis (GCMS) is attempted for the methanol extract of dried leaves for the exploration of most phytochemicals in a quantitative manner. The plant possesses various medicinal properties as per the report on Indian medicinal plants[15]. So the motto of our study is to identify the most phyto-compounds in the methanolic extract of *Pergularia daemia* leaves along with their concentrations by GC-MS analysis.

EXPERIMENTAL

Plant Material Collection

The *Pergularia daemia* plant leaves were collected at Nallamalla forest, Prakasam district, Andhra Pradesh in August. The plant was properly identified by a senior Botanist Prof. Dr. Vatsavaya S. Raju, Department of Botany, Kakatiya University, Telangana-506 009.

Preparation of extracts

The air dried powder of leaf was extracted in a soxhlet extractor with methanol and was air dried in a hot air oven at 40 degrees Celsius then the material was softened by soaking (maceration) in hot water with occasional steering for 16 h and water extract was filtered. Finally, the solvent extract was evaporated to remove the final traces of the concerned solvent. The extract recovery in the solvent was expressed as a percent of the plant sample dry matter. This extract was utilized for the GC-MS examination to identify the components.

GC-MS (Gas Chromatography-Mass Spectrometry) analysis

To the sample, 500 µL of n-butane was added and vortex for 1 min and poured into a screw-capped GC glass vial. Next, the sample was exposed to GC-MS analysis for metabolic data at 70eV with an electron impact ionization (EI^+) source. The source is GC 7890 and MS of 5977N, Agilent Technologies, palo Alto, CA, USA. The capillary HP-5 MS column with dimensions 30mX 250 µmi.d. X 0.25 µm film thickness) consisting of 5% phenyl 95% methylpolysiloxane as stationary phase in splitless mode was used for analysis. The investigation was performed by injecting an aliquot of 1µL of the extract into the injection port at 25°C temperature by the use of Helium as flow gas with an overall flow rate of 1 mL/minute. In this study the oven temperature was programmed as-initial oven temp set at 50°C for 2 min then elevated to 150°C at a rate of 50°C/min and maintained for 2 min then finally raised to 300°C at a rate of 15°C/min, where it was held for 20 minute.

Data Pre-Processing

The individual components from the peaks obtained in GC-MS spectra were recognized by relating their mass spectra with the spectra of

known compounds stored in the spectral catalog of NIST library (version 8.0). The baseline correction, smoothing, noise reduction and integration were done before the identification.

RESULTS AND DISCUSSION

GC-MS is one of the leading techniques to recognize various constituents of crude extracts like branched chain, long chain, hydrocarbons, amides, vitamins, acids, alcohols, esters, etc. So, in this study GC-MS analysis was selected to identify the components of based on retention time, peak area, molecular formula, molecular weight and nature of compounds were also analyzed from NIST data. The separated components with their proper Retention times (RT), peak area in percentage, molecular formula, and molecular weight (MW) obtained as per GC-MS analysis Fig. 1 of the methanol extract of Pergularia daemia leaves were presented Table 1.

Table 1: Compounds recognized in methanolic extract of P. daemia leaves in GC-MS

S.No.	Retention time	Peak area %	Name of the compound	Molecular Formula	Molecular Weight	Nature of the compound
1	3.79	0.31	1-Dodecene	$C_{12}H_{24}$	168	Alkene
2	4.19	0.09	Trans–alpha–Bergamotene,	$C_{15}H_{24}$	204	Bicyclic compound
3	4.71	0.16	Pentadecane	$C_{15}H_{32}$	212	Alkane
4	5.57	0.88	Dichloroacetic acid, 4-hexadecylester	$C_{18}H_{34}Cl_2O_2$	353.4	Acid ester
5	6.85	0.17	Tetratriacontane	$C_{34}H_{70}$	478	Alkane
6	7.29	0.04	Heptacosane	$C_{27}H_{56}$	380	Alkane
7	7.60	1.21	1- octadecene	$C_{18}H_{36}$	252	Alkene
8	8.12	0.10	6-Octen-1-ol, 3,7-dimethyl-,(R)-	$C_{10}H_{20}O$	156	Terpenoids
9	8.69	0.12	Nonadecane	$C_{19}H_{40}$	268	Alkane
10	9.11	0.18	Dibutyl phthalate	$C_{16}H_{22}O_4$	166	Aromaticcarboxylic acid ester
11	9.58	1.68	E-15-Heptadecenal	$C_{17}H_{32}O$	252	Fatty aldehyde
12	10.59	0.42	Heneicosane	$C_{21}H_{44}$	296	Acyclic alkanes
13	11.07	0.24	Ethyl Oleate	$C_{20}H_{38}O_2$	310	Fatty acid Ester
14	11.42	1.76	1-Docosene	$C_{22}H_{44}$	308	Alkene
15	12.36	2.18	1-Iodo-2-methylundecane	$C_{12}H_{25}I$	296	Iodoalkane
16	13.14	1.74	Dichloroacetic acid, heptadecyl ester	$C_{19}H_{36}Cl_2O_2$	367	Acid ester
17	14.03	8.46	Pentacosane	$C_{25}H_{52}$	352	Alkane
18	14.74	2.36	Hexacosane	$C_{26}H_{54}$	336	Alkane
19	15.57	15.89	Heptacosane	$C_{27}H_{56}$	380	Alkane
20	16.28	4.25	Squalene	$C_{30}H_{50}$	410	Triterpene
21	16.92	7.87	Nonacosane	$C_{29}H_{60}$	408	Alkane
22	17.55	3.13	Tridecane, 7-hexyl-	$C_{19}H_{40}$	268	Alkane
23	18.35	3.17	Hentriacontane	$C_{31}H_{64}$	436	Alkane
24	19.9	2.50	9-Hexacosene	$C_{26}H_{52}$	364	alkene
25	19.48	1.44	(Z)-14-Tricosenyl Formate	$C_{24}H_{46}O_2$	366	ester
26	20.19	1.71	Z-12-Pentacosene	$C_{25}H_{50}$	350	Alkene
27	20.48	1.49	8-Hexadecenal,14-methyl-(z)-	$C_{17}H_{32}O$	252	aldehyde
28	21.44	7.74	1-Hexacosanol	$C_{26}H_{54}O$	382	alcohol
29	21.84	1.47	Beta-Sitosterol acetate	$C_{29}H_{48}$	396	Steroid
30	22.72	1.82	1-Docosanethiol	$C_{22}H_{46}S$	342	Fatty Thioalcohol
31	23.42	2.49	Phytol	$C_{20}H_{40}O$	296	Terpenoid
32	24.33	2.21	Cyclotriacontane	$C_{30}H_{60}$	420	Cycloalkane
33	25.06	0.97	Tricosane	$C_{23}H_{48}$	324	Alkane
34	25.99	1.46	Heptanoic acid, phenyl methyl ester	$C_9H_{14}O_2$	130	Ester
35	26.72	1.87	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	C_6H_{12}	84	Alkane
36	27.19	1.03	1,19-Eicosadiene	$C_{20}H_{38}$	278	Terminal alkene
37	27.77	0.64	Octanoic acid, octadecyl ester	$C_{26}H_{52}O_2$	396	Ester
38	28.30	2.56	Undecanoic acid, phenyl methyl ester	$C_{18}H_{28}O_2$	276	Ester
39	29.38	0.52	2-methyl- 2-docosene	$C_{23}H_{46}$	322	Alkene
40	29.63	0.43	Propanamide, N-(4-methoxyphenyl)-2,2,3,3,3-pentafluoro-	$C_{10}H_8F_5NO_2$	193	Amide
41	29.97	0.48	Androst-5,7-dien-3-ol-17-one,acetate	$C_{22}H_{32}O_4$	360	Steroid
42	30.50	0.85	2-Propenamide,3-phenyl-N,N- bis(phenylmethyl)-	$C_{23}H_{21}NO$	327	Amide
43	31.05	1.57	Butanoic acid, 2-methyl-	$C_5H_{10}O_2$	88	Fatty acid
44	31.90	0.39	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-	$C_{14}H_{22}O_2$	222	Catechol
45	32.25	0.54	Pyridine-3-carboxamide,Oxime, N-(2-trifluoromethylphenyl)-	$C_{13}H_{10}F_3N_3O$		Vitamin
46	32.65	0.68	Octadecane, 1-(ethenyloxy)-	$C_{20}H_{40}O$	296	Ether
47	33.00	0.60	2,6-methano-3-benzazocin-8-ol	$C_{17}H_{23}NO$	257	Phenolic
48	33.58	1.09	Octanoic acid, Hexadecyl ester	$C_{24}H_{48}O_2$	368	Esters
49	34.42	2.61	Dodecanoic acid, phenylmethyl ester	$C_{19}H_{30}O_2$	290	Ester
50	36.21	1.82	2-Benzo [1,3] dioxol-5-yl-8-methoxy-3-nitro-2H-chromene	$C_{17}H_{13}NO_6$	327	chromene
51	36.70	0.61	Androst-5-ene-3,17-diol, 4,4-di…	$C_{19}H_{26}O_2$	286	Steroid

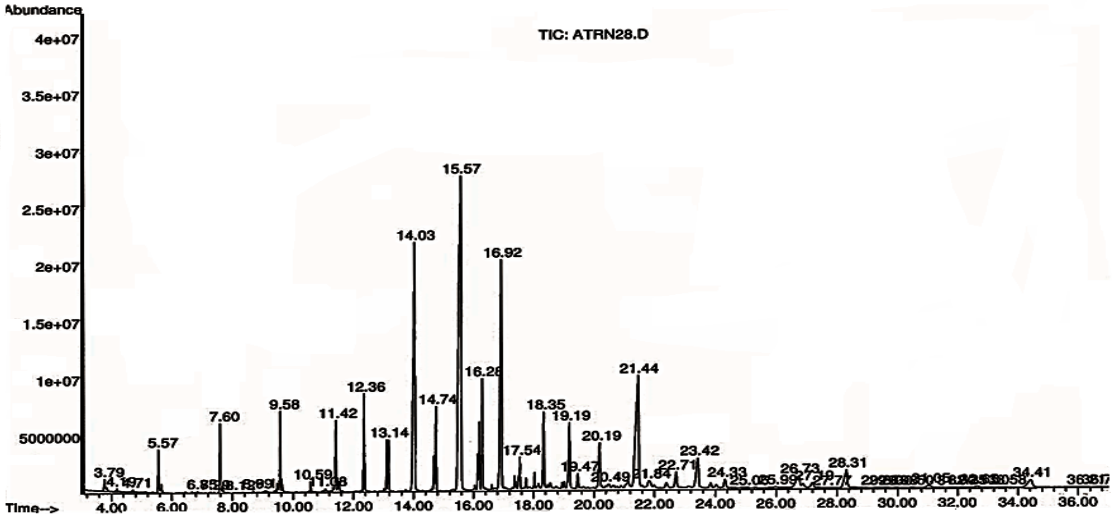


Fig. 1. Graphical presentation of GC-MS spectral chromatogram of methanolic leaf extract

As per the NIST library, the leaf extract contains several hydrocarbons such as alkanes, alkenes; fatty acids, alcohols, esters, ethers, amides, steroids, terpenoids, triterpenes, vitamin, chromenes and various phenolic compounds. Further as per the knowledge of the author on the reports of *P. daemia*, the compounds Pentadecane, Tetratriacontane, Dibutyl phthalate, 1-(ethenyloxy)-Octadecane and Squalene were not reported in any GC-MS analysis and some are known to be biologically important Table 2.

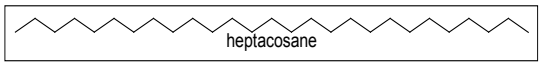


Fig. 2. Structure of the major compound identified in GC-MS of leaf extract

Further, the mass spectrum of the major component Fig. 2. Separated at a retention time of 15.57 min with peak area of 15.89% was also studied and reported in Fig. 3. After heptacosane the compound pentacosane was separated as major component at 14.03 with peak area 8.46%.

The identified compounds were of many biological importance, the details of some of the components were presented in Table 2. GC-MS examination of phytochemicals in plants provides the design of the pharmacological importance of that plant. So, this type of study with GC-MS examination is the leading step towards knowing the type of medicinal values in the concerned medicinal plants and helpful for detailed study.

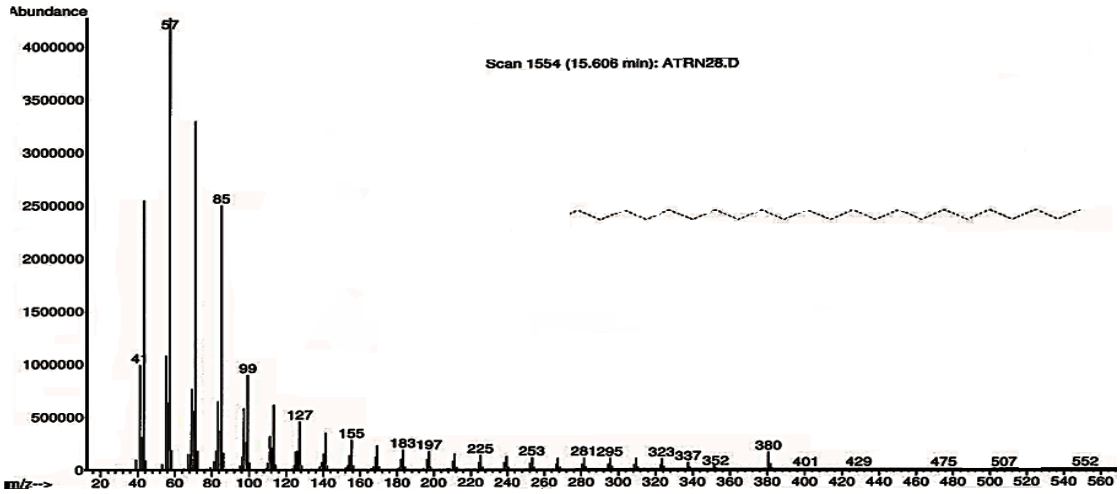
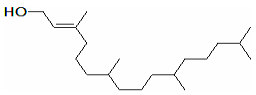
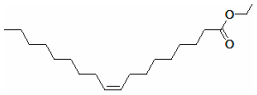
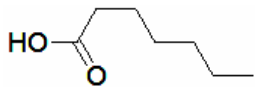
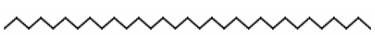
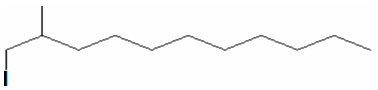
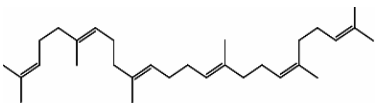
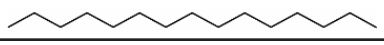


Fig. 3. Mass spectrum of the major compound identified in GC-MS of leaf extract

Table 2: Bioactivities of some phytocompounds identified in methanolic extract of *P.daemia* leaves by GC-MS

S.No	Compound Name	Structure	Biological activity	Referance
1	Phytol		Antinociceptive, Antioxidant	16
2	Ethyl oleate		Antibacterial	17
3	Heptanoic acid		Analgesic	16
4	Nonadecane		Antimicrobial and Anticancer	18
5	Nonacosane		Antibacterial	19
6	Hentriacontane		Anti-inflammatory, Antioxidant, Antitumor	20
7	Tricosane		Anti bacterial	19
8	1–Iodo 2-methylundecane		Antimicrobial	21
9	Heptacosane		Anti bacterial	19
10	Pentacosane		Anti bacterial	19
11	Squalene		Antibacterial, Antioxidant, Antitumor, Cancer preventive immunostimulant, lipoxygenase inhibitor, pesticide.	21
12	Tetratriacontane		Antibacterial and Antfungal	22
13	Pentadecane		Antibacterial	19

CONCLUSION

A total of 51 components were identified in the complete GC-MS examination of *P.damea* leaves extract. The existence of various bioactive compounds reported in this GC-MS analysis for the methanolic extract of leaves of *P. daemia* confirmed the use of leaf extract for various activities by the traditional practitioner. Always isolation of individual phytochemicals and subjecting them to various biological activities will surely give effective results that open a new area of examination of the components and their pharmacological efficacy. From these results, it could be concluded that "Pergularia daemia" contains various bioactive compounds. Therefore, the plant is recommended as a plant with good phytopharmaceutical rank.

ACKNOWLEDGEMENT

The authors are thankful to Acharya Nagarjuna University for constant support and encouragement.

Conflict of interest

The authors declare no conflict of interest.

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