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GC-MS-MS analysis of *Trichilia connaroides* (Wight & Arn.) Bentv (Meliaceae): A tree of ethnobotanical records

***N. Senthilkumar, S. Murugesan and K. B. Vijayalakshmi**

Division of Bioprospecting, Institute of Forest Genetics and Tree Breeding, R.S. Puram, Coimbatore, India

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ABSTRACT

GC-MS-MS analysis was carried out for the identification of the phytochemicals present in the methanolic leaf extract of Trichilia connaroides. It indicates that the leaf extract is rich in phenolics, fatty acids, flavonoids and antioxidants. Many phytochemcial compounds such as Naphthalene, Palmitic acid, Oleic acid, Isotridecanol, phytol, Lycopersen, Isochiapin B and Serverogenin acetate have been identified. These compounds have been found to possess anti-insect, antimicrobial, antioxidant, anticancer, and antiulcerogenic activities.

Keywords: GC-MS analysis, phytochemicals, methanol extract and *Trichilia connaroides.*

INTRODUCTION

Trichilia connaroides (Wight & Arn.) Bentv is known to possess biological and pharmacological activities [1] and has long been used as a traditional remedy in India and China [2-5]. It is a small evergreen tree widely distributed in South and East Asia such as India, Indonesia, and China. The bark and leaves of *T. connaroides* are bitter with tonic properties [1]. According to Indian folklore, a decoction of the leaves is taken to treat cholera [6]. Its roots are used in Chinese medicine to treat arthritis, tonsillitis, and other ailments [3]. Nepalese use the fixed oil from seeds for lighting lamps [6]. Indian people use the wood in the manufacturing of beams and agricultural implements. It is used as a pesticidal plant in southern China [4]. Different solvent extracts of *T. connaroides* were found effective against agriculturally important insect pests namely, *Spilosoma obliqua, Spodoptera litura Amsacta albistriga*, and *Plutella xylostella* in terms of larval mortality [7-9]. The main chemical constituents of *T. connaroides* are the bitter principles limonoids. Compounds belong to this group exhibit a range of biological activities including insecticidal, antifeedant and growth regulating activities as well as antibacterial, antifungal, antiviral, antimalarial and anticancerous activities on humans [10]. In our preliminary investigation, leaf extract of *T. connaroides* was found effective against Ailanthus defoliator, *Eligma narcissus indica* Cramer and teak defoliator, *Hyblaea puera* Cramer. Hence, the present study aimed at identification and characterization of secondary metabolites from *T. connaroides* for insecticidal properties show an insight into the novel bio insecticides.

MATERIALS AND METHODS

Collection of plant material

Fresh leaves of *T. connaroides* were collected from Yercaud located between 11º46’46” N and 11º77’94” N latitudes and 78º12’12” E 11º77’77” E longitudes in the Shevaroys range of hills in Eastern Ghats, Salem district, Tamilnadu. It is situated at an altitude of 1515 metres above sea level. The above said plant material was collected

by interaction with the hill tribes in Yercaud, *Vellalas.* The collected plant material was authenticated by a taxonomist at IFGTB, Coimbatore.

Preparation of plant extracts

The collected plant materials were air dried and ground into uniform powder. Dry powder of plant sample was extracted with methanol using soxhlet apparatus for 6 hours. The extract was filtered over anhydrous sodium sulphate followed by concentrated using rotary evaporator. The concentrated extract was subjected to freeze drying in a lyophilizer till dry powder was obtained. Finally the extracted powder was resuspended with the methanol at the concentration of 100mg/ml (w/v) followed by filtration through Varian Bond Elut C_{18} solid phase extraction to remove impurities. 1µl of this solution was employed for GC-MS-MS analysis.

GC-MS-MS analysis

The GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS with Fused silica 15m x 0.2 mm ID x 1µm of capillary column. The instrument was set to an initial temperature of 110 ºC, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 ºC, at the rate of an increase of 5 ºC/min, and maintained for 9 min. Injection port temperature was ensured as 250 ºC and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

Identification of phytocompounds

Interpretation on mass-spectrum GC-MS-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

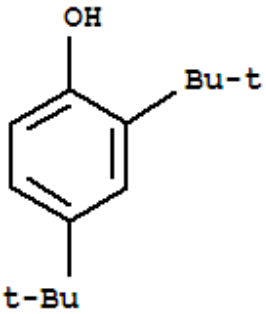
RESULTS AND DISCUSSION

The GC-MS-MS study of *T. connaroides* leaves has shown many phytochemicals which contribute to the biological activity of the plant. The active principles, with their retention time (RT), molecular formula, molecular weight (MW) and the concentration (peak area %) are presented in table 1. The GC- MS Chromatogram of the number of peaks of the compounds detected was shown in fig.1. The total number of compounds identified in methanol extract was 40.

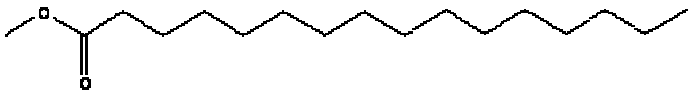
Table 1. GC-MS analysis of methanol leaf extract of *T. connaroides*

S.No	Retention time (min)	Name of the Compound	Peak Area %	Molecular Weight	Molecular Formula
1	5.457	Methyl 1 1',13'-dioxo-12'-aza-[4,4,3]-pro	5.14	325	$C_{19}H_{19}No_4$
2	5.609	4a,8a-(methaniminomethano)naphthalene-9,11	3.33	275	$C_{18}H_{13}No_2$
3	6.022	Naphthalene	8.47	128	$C_{10}H_8$
4	6.562	7-Tetradecene	0.89	196	$C_{14}H_{28}$
5	6.990	Bicyclo[3.1.1]heptan-3-one,2,6,6-trimet	1.64	152	$C_{10}H_{16}O$
6	7.473	Isotridecanol	2.44	200	$C_{13}H_{28}O$
7	7.623	Octane	1.70	114	C_8H_{18}
8	8.603	1-bromo-2-chloro-1,1-difluro-2-tridecan (Hexacosane)	1.93	330	$C_{13}H_{22}BrClF_2$
9	9.024	Tetrahydroxy myrcenol	4.82	158	$C_{10}H_{22}O$
10	9.742	Dodecyl acrylate(Oleic acid)	0.78	240	$C_{19}H_{36}O_2$
11	9.868	Benzene,1-(1,5-dimethyl-4-hexyl-4-methyl	5.25	202	$C_{15}H_{22}$
12	10.032	17-pentatriacontane	1.86	490	$C_{35}H_{70}$
13	10.182	Phenol,2,4-bis(1,1-dimethylethyl)	5.23	206	$C14H_{22}O$
14	10.448	Silane-trichlorodocosyl	1.76	442	$C_{22}H_{45}Cl_3Si$
15	10.716	2-Undecanethiol,2-methyl	1.62	202	$C_{12}H_{26}S$
16	10.865	Pentadecane	1.82	212	$C_{15}H_{32}$
17	10.988	Undecane	1.64	156	$C_{11}H_{24}$
18	11.078	Decane	1.79	142	$C_{10}H_{22}$
19	11.318	Hydroxylamine,o-decyl	2.06	173	$C_{10}H_{23}No$
20	11.628	Dodecanoic acid	1.55	200	$C_{12}H_{24}O_2$
21	12.103	2-Hexyl-1-octanol	1.56	214	$C_{14}H_{30}O$
22	13.374	Germacrane-B	1.2	210	$C_{15}H_{30}$
23	13.389	Erucic acid	0.59	338	$C_{22}H_{42}O_2$
24	14.392	Phthalic acid, cyclobetyl octyl ester	2.51	332	$C_{20}H_{28}O_4$
25	14.961	Nonadecane	1.25	268	$C_{19}H_4O$
26	15.187	Hexadecanoic acid methyl ester (palmitic acid)	13.44	270	$C_{17}H_{34}O_2$
27	15.256	Serverogenin acetate	1.81	544	$C_{29}H_{36}O_{10}$
28	15.256	Dotriacontane	1.62	450	$C_{32}H_{66}$
29	15.256	Isochiapin B	1.30	346	$C_{19}H_{22}O_6$
30	15.256	Phytol	0.43	296	$C_{20}H_{40}O$
31	16.052	Oleic acid	3.57	282	$C_{18}H_{34}O_2$
32	18.345	Ethyl linoleate	0.76	308	$C_{20}H_{34}O_2$
33	18.427	Ethyl oleate	1.39	310	$C_{20}H_{38}O_2$
34	20.762	2-(3-Innoxyl 3-5-aminopyridol (2,3-dipyrimidine)	3.49	261	$C_{15}H_{11}N_5$
35	20.990	O,O'- Biphenol,4,4',6,6'-Tetra-Butyl	0.46	410	$C_{28}H_{42}O_2$
36	21.711	Eicosane	0.71	282	$C_{20}H_{42}$
37	23.657	Phthalic acid octyl tridec-2-yn-1-yl ester	1.45	456	$C_{29}H_{44}O_7$
38	27.482	6,10,14,18,22-Tetra Cosa pentane 2-ol, 3-Bromo,2,6,10,15	3.56	506	$C_{30}H_{51}BrO$
39	27.485	Solanesol	1.73	630	$C_{45}H_{74}O$
40	27.481	Lycopersen	1.35	546	$C_{40}H_{66}$

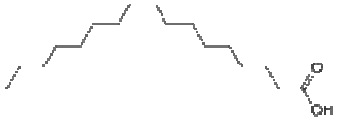
Most of the compounds are not earlier reported from *T. connaroides* leaves [1]. Biological activities of most of the compounds such as Naphthalene, Palmitic acid, Oleic acid are well known as anti-insect, anti-microbial agents [11].



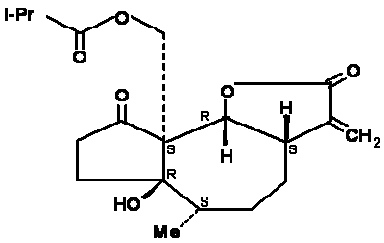
Phenol,2,4-bis(1,1-dimethylethyl)



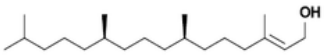
Palmitic acid



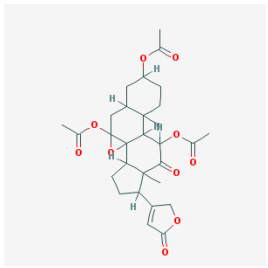
Oleic acid



Isochiapin B

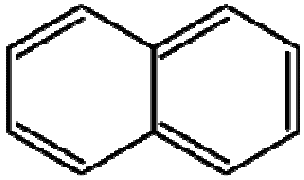


Phytol

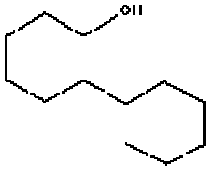


Serverogenin acetate

Tetrahydroxy myrcenol



Naphthalene



Isotridecanol

The presence of Hexadecanoic acid (13.44%) indicates that the leaf extract of *T. connaroides* may be used as potential antifeedant agents against insects [11]. Some of the compounds such as Isotridecanol, phytol, Lycopersen, Isochiapin B and Serverogenin acetate are known for their application in pharmacology [11-12].

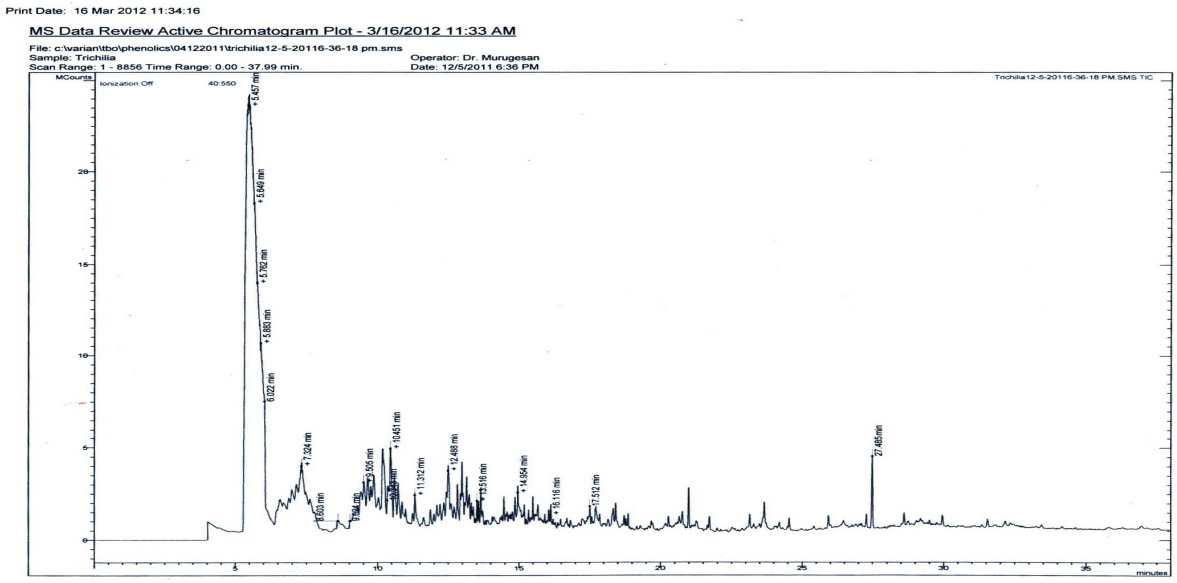


Fig.1. GC-MS chromatogram of *Trichilia connaroides* methanolic extract

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