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Bioactive Compounds in Leaves of *Corchorus trilocularis* L. BY GC-MS Analysis

Dhanalakshmi R*, Manavalan R

Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar 608002, Chidambaram, Tamilnadu, India

*Corres.author: dhanama@gmail.Com, Tel: 09894309491

Abstract: In the plant kingdom, there are thousands of plants that yield medicines or drugs useful to man. The plant *Corchorus trilocularis* belonging to the family Tiliaceae form the part of the traditional medicine system. The species of this family possess cholesterol lowering activity, anti-inflammatory and demulcent. The modern methods describing the identification and quantification of active constituents in the plant materials may be valuable for proper standardization of herbals and its formulations. Therefore, the present investigation was carried out to determine the possible bioactive compounds present in the leaves of the plant. The ethanol extract of leaves of *Corchorus trilocularis* was subjected to Gas Chromatography and Mass Spectrometry analysis revealed the presence of twelve phyto compounds.

Keywords: Corchorus trilocularis; ethanol extract; GC-MS analysis; bioactive constituents.

Introduction

Plants owing to its medicinal value have continued to play a dominant role in the maintenance of human health. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of world population [1]. Plants still represent a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs [2]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Traditional systems of medicines are prepared from a single plant or combinations of number of plants. The efficacy depends on the use of proper plant part and its biological potency which in turn depends upon the presence of required quantity and nature of secondary metabolite in a raw drug [3-4]. Several Pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Pure drugs that are produced or isolated from plants may be chosen for their high activity against a human disease, but they have disadvantages. They rarely have the same level of activity as the crude extract at parallel dose or concentrations of the active component [5]. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards [6]. As a result of the present situation there is a need of essential effort to standardize the plant materials. Corchorus trilocularis L. (Tiliaceae) is one of the most common plants in India and is available throughout the year. The plant has been reported to possess anti-inflammatory [7] and demulcent [8]. In traditional folklore medicine in India, Corchorus trilocularis is also used for the treatment of syphilis [9]. But there have been no reports on the bioactive constituents from ethanol extract of leaves of Corchorus trilocularis Linn. Hence, the present study is to determine the bioactive constituents present in the ethanol extract of leaves of Corchorus trilocularis by Gas chromatography and Mass spectroscopy (GC-MS) technique.

Materials and Methods

Collection and Identification of the Plant material:-

Fresh leaves of the selected plant Corchorus trilocularis were collected from Thirunelveli district,

Tamil Nadu, India. The plant materials were taxonomically identified and authenticated by Dr. V. Chelladurai, Research officer - Botany (scientist C), Central council for research in Ayurveda and Siddha, Govt. of India; Thirunelveli. The plant was thoroughly washed in running tap water to remove soil particles and adhered debris and finally washed with distilled water. The leaves of the plant alone were segregated and dried under shade, pulverized by a mechanical grinder into fine powder. The powdered materials were stored in air tight polythene bags till use.

Preparation of extracts

The powdered plant materials were extracted with ethanol (99.9%). The extraction was done by hot continuous percolation method in Soxhlet apparatus for 24 hrs [10]. The extract was concentrated by using a rotary evaporator till dry powder was obtained. The final residue thus obtained was then subjected to GC-MS analysis [11].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The ethanol extract of *Corchorus trilocularis* leaves was analyzed through GC-MS for the identification of different compounds. The GC-MS analysis was carried out by using Clarus 500 (Perkin - Elmer) Gas chromatograph equipped and coupled to a mass detector Turbo mass gold (Perkin - Elmer) spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane, 30m x 0.25 mm x 0.25 μ m df) of capillary column. The oven was set to an initial temperature 110°C for 2 min, further increased up to 200 °C at the rate of 10 ° C/min. Finally temperature was raised up to 280°C, at the rate of 5°C /min for 9 min. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1 ml/min. An aliquot of 2µl of sample was injected into the column with the injector temperature at 250°C (Split ratio of 10:1). The electron ionization system with ionizing energy of 70 eV was used. Mass spectral scan range was set at 45-450 (m/z). Total GC running time was 36 minutes.

Identification of compounds

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technique (NIST Version-Year 2005) having more patterns. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The spectrum of the unknown compound was compared with the spectrum of the compound stored in the NIST data library (version 2005). The name, molecular weight, molecular formula and structure of the components of the test material were obtained.

Results:

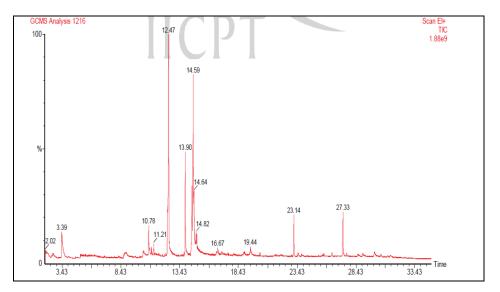


Fig. 1: The GC - MS Chromatogram of ethanol extracts of leaves of Corchorus trilocularis

GC–MS chromatogram of the ethanol extract of leaves of *Corchorus trilocularis* (Fig. 1) showed twelve peaks indicating the presence of twelve phytochemical compounds. The mass spectra of identified compounds were matched with those found in the NIST/NBS spectral database given in table. 1. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. Table 2 shows the nature and biological activity of the predicted compounds. The individual fragmentation patterns of necessary compounds were illustrated in figures A-J.

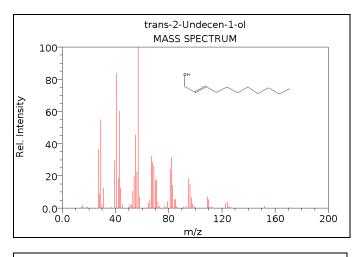
No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1	3.39	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144	8.70
2.	10.78	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.25
3.	11.03	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170	0.65
4	11.21	E-7-Tetradecenol	$C_{14}H_{28}O$	212	1.23
5	12.47	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	34.69
6	13.90	Phytol	$C_{20}H_{40}O$	296	10.78
7	14.59	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	26.72
8	14.82	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368	2.31
9	16.67	9-Tetradecen-1-ol, acetate, (E)-	$C_{16}H_{30}O_2$	254	1.02
10	19.44	10-Methyl-E-11-tridecen-1-ol propionate	$C_{17}H_{32}O_2$	268	1.00
11	23.14	Squalene	C ₃₀ H ₅₀	410	3.98
12	27.33	Vitamin E	$C_{29}H_{50}O_2$	430	5.66

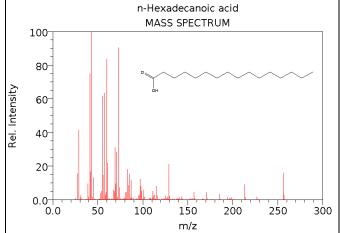
Table 1: Phytochemical compounds identified in ethanol extract of leaves of Corchorus trilocularis

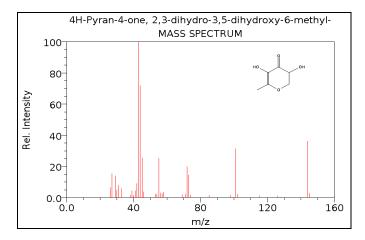
 Table 2: Biological activities of phytochemical compounds identified in ethanol extract of leaves of

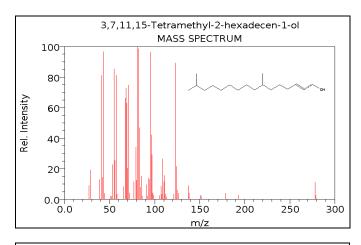
 Corchorus trilocularis

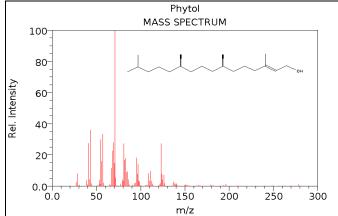
No.	Name of the compound	Compound Nature	Biological Activity
1	4H-Pyran-4-one, 2,3-dihydro-3,5-		Anti microbial, Anti-inflammatory and anti
	dihydroxy-6- methyl-	Flavonoid	proliferative activity
2	3,7,11,15-Tetramethyl-2-hexadecen-1-	Terpene	Anti microbial, Anti-inflammatory activity
	ol	Alcohol	
3	trans-2-Undecen-1-ol	Alcohol	No activity reported
4	E-7-Tetradecenol	Alcohol	Anti microbial activity
5	n-Hexadecanoic acid		Lubricant, anti-androgenic flavor,
		Palmitic	hypocholesterolemic, flavor, hemolytic,
		acid	antioxidant, pesticide, 5-alpha reductase
			inhibitor
6	Phytol	Diterpene	Hypocholesterolemic, Antimicrobial,
			Anticancer, Cancer preventive, Diuretic Anti
			inflammatory
7	9,12,15-Octadecatrienoic acid, methyl	Fatty acid	Anti inflammatory, Hypocholesterolemic,
	ester, (Z,Z,Z) -	ester	Cancer preventive, Hepatoprotective,
		compound	Nematicide, Insectifuge
8	Docosanoic acid, ethyl ester	Behenicacid	No activity reported
		ethyl ester	
9	9-Tetradecen-1-ol, acetate, (E)-	Acetate	No activity reported
10		compound	
10	10-Methyl-E-11-tridecen-1-ol	Alcoholic	Antimicrobial activity
	propionate	compound	
	Squalene	Triterpene	Antibacterial, Antioxidant, Pesticide,
11			Antitumor, Cancer preventive,
	· · · · ·		Immunostimulant, Chemo preventive,
12	Vitamin E	Vitamin	Antiageing, Analgesic, Antidiabetic, Anti
			inflammatory, Antioxidant, Antileukemic,

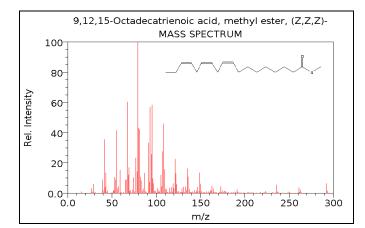


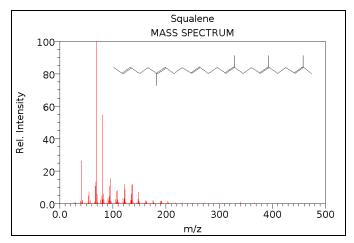


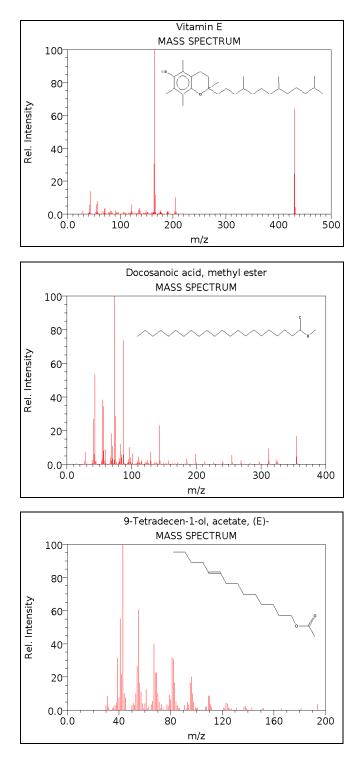












(Fig A-J: The individual fragmentation pattern of the important compounds)

Discussion

The GC/MS analysis showed that twelve compounds were present in ethanol extract of leaves of *Corchorus trilocularis*. The fragmentation pattern of the major compound is n-Hexadecanoic acid, retention time is 12.47 and peak area percentage is 34.69. n-Hexadecanoic acid - palmitic acid possess an antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic activity. The compound 10-Methyl-E-11-tridecen-1-ol propionate - with retention time and peak are percentage were 19.44 and 1.00 respectively. This compound can be act as antimicrobial agents [12]. The compound 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- retention time is 14.59 with 26.72 peak area percentage. 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-Fatty acid ester compound posses an anti inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective activity. [13]. Phytol was also detected with retention time 13.90 and peak area percentage 10.78. This compound is suggested to be a diterpene compound and it might act as a hypocholesterolemic,

antimicrobial, anticancer, diuretic and anti inflammatory agents. Trans-2-Undecen-1-ol was detected 0.65 % relative amount with 11.03 retention time. E-7-Tetradecanediol was detected with retention time and peak area percentages were 11.21 and 1.23 respectively. E-7-Tetradecenol - alcohol act as an anti microbial agent. Squalene was also detected with retention time 23.14 and peak area percentage 3.98. Squalene is suggested to be a tri-terpene compound and it may act as an antibacterial, antioxidant, antitumor, immunostimulant, chemopreventive, lipoxygenase inhibitor and anti HIV. Vitamin E was detected with retention time 27.33 and peak area percentage 5.66. It is a vitamin compound and it may acts as an antiageing, analgesic, antidiabetic, anti inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, antiulcerogenic, antispasmodic, antibronchitic and anticoronary, hypocholesterolemic, vasodilator and hepatoprotective [14,15,16]. The compound 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl- flavonoids has retention time 3.39 and peak area percentage 8.70 possess anti microbial, anti-inflammatory and anti proliferative activity[17]. 3,7,11,15-Tetramethyl-2-hexadecen-1-ol -Terpene was detected 3.25 % related amount with 10.78 retention time also posses anti microbial and anti-inflammatory activity [18].

Conclusion

In the present study, twelve phytochemical constituents have been identified from the ethanol extract of leaves of *Corchorus trilocularis* by Gas Chromatogram - Mass Spectrometry (GC - MS) analysis. The presence of these phytochemical constituents justifies the use of this plant for various ailments by traditional practitioners. Isolation of individual photochemical constituents and subjecting it to biological activities are being undertaken.

Acknowledgement

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