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GC-MS Analysis of Phytocomponents in the Methanolic Extract of *Eupatorium triplinerve*

Christy Selvamangai[1], Anusha Bhaskar[2*]
[1]Department of Biotechnology, Alpha Arts and Science College, Chennai.
[2]Department of Biotechnology, PRIST University, Vallam, Thanjavur 614 403.

Abstract

Objective: To characterize the phytochemical constituents of *Eupatorium triplinerve* using GC – MS.

Methods: Ten grams of the powdered sample was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethanol and methanol respectively. n-hexane and chloroform did not elute much of the compounds. The methanol fraction of the *Eupharbatum triplinerve* was taken for GC-MS analysis. The analysis was carried out on a GC Clarus 500 GC system with a column packed with Elite – 1 (10% dimethyl poly siloxane, 30 x 0.25 mm ID x 1 EM df), the compounds are separated using with Helium as carrier gas at a constant flow 1ml/min. sample extract (2 µL) injected into the instrument was detected by Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software.

Results: The GC MS analysis provided peaks of eleven different phytochemical compounds namely hexadecanoic acid (14.65%), 2,6,10-trimethyl,14-ethylene-14-pentadecne (9.84%), Bicyclo[4.1.0]heptane, 7-butyl- (2.38%), Decanoic acid, 8-methyl-, methyl ester (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1,14-tetradecanediol (6.78%), Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (19.18%) and 2-hydroxy-3-[(9E) -9-octadecenoyloxy] propyl(9E)-9-octadecenoate (8.79%).

Conclusion: The bioactive compounds in the methanolic extract of *Eupatorium triplinerve* have been screened using this analysis. Isolation of individual components would however, help to find new drugs.

*Corresponding author, Mailing address:
Anusha Bhaskar
Email: dranushaparthiban@gmail.com

Key words:
GC-MS analysis, *Eupatorium triplinerve*, phytocomponents, hexadecanoic acid.

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Introduction

In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the World Health Organization (WHO) states that around 85 – 90% of the world's

population consumes traditional herbal medicines [1]. Plants are capable of synthesizing an overwhelming variety of low-molecular weight organic compounds called secondary metabolites, usually with unique and complex structures. Many metabolites have been found to possess interesting biological activities and find applications, such as pharmaceuticals, insecticides, dyes, flavors and fragrances.

Eupatorium triplinerve Vahl is familiarly known as Ayappana belongs to Asteraceae family. It is a slender herb with narrow lanceolate leaves and large number of pedicelled flower-heads at the top of the branch. The methanolic extract of *E triplinerve* is reported to have hepatoprotective effect and antioxidant effect against carbon tetracholoride induced hepatotoxicity in rats [2], while the ethanolic extract had analgesic effect in inflammatory model of pain [3], antibacterial and antifungal activity [4], antiseptic and in the treatment of various ulcers and haemorrhages [5]. Although the plant is used in Ayurvedic medicine for the treatment of ailments there are no reports on the constituents that are responsible for the therapeutic effect. With this background the present study was aimed to identify the phytoconstituents present in *E triplinerve* using GC-MS analysis.

2. Materials and methods

2.1 Collection and preparation of plant material

Fresh plants of *E. triplinerve* were collected from the natural habitats of Tiruchirappalli, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and other adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

2.2 Plant sample extraction

Plant sample extraction and Column chromatography

Ten grams of powdered sample was extracted with 50 mL methanol overnight and filtered through ash less filter paper with sodium sulphate (2 g). The crude extract was subjected to column chromatography over silica gel (100-200 mesh) and eluted with n-hexane, chloroform, ethanol and methanol respectively. n-Hexane and Chloroform did not elute much of the compounds. The methanol fraction of the *Eupharbatum triplinerve* was taken for GC-MS analysis.

Gas Chromatography- Mass Spectrum Analysis (GC-MS)

GC-MS analysis was carried out on a GC Clarus 500 Perlin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrophotometer (GC – MS) instrument employing the following conditions: column Elite – 1 fused silica capillary column (30 x 0.25 mm ID x 1 EM df, composed of 100% Dimethyl polysiloxane), 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 EI was employed (split ratio of 10:1 injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min). with an increase of 10 C/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 0.5s and fragments from 40 to 550 Da.

Identification of components

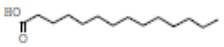
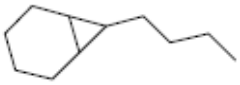
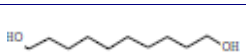
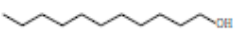
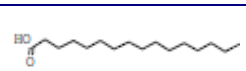
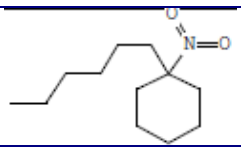
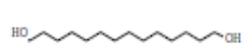
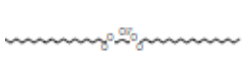
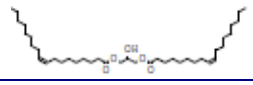
Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the

known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

Results

The identified compounds of the leaves of *E triplinerve*, their retention indices, percentage composition, chemical structure and activities are given in Table 1. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases. The results showed the presence of hexadecanoic acid (14.65%), 2,6,10-trimethyl, 14-ethylene-14-pentadecne (9.84%), bicycle[4.1.0]heptanes (2.38%), decanoic acid (3.86%), 1-undecanol (7.82%), 1-hexyl-1-nitrocyclohexane (2.09%), 1,14-tetradecanediol (6.78%), octadecanoic acid (19.18%) and 2-hydroxy-3-[(9E)-9-octadecenoyloxy]propyl(9E)-9-octadecenoate (8.79%). The spectrum profile of GC-MS confirmed the presence of 10 major components with retention time15.084, 15.75, 16.2,16.40,16.96,17.15,18.38,19.986,20.148 and 21.619 respectively (Figure 1). The individual fragmentation of the components is illustrated in (Figures 2A-2J).

Table1: Phytocomponents identified in the methanolic leaf extract of *Eupatorium triplinerve* by GC-MS

No	RT	Name of the compound	Molecular formula	Molecular weight	Peak area %	Structure	Nature of compound	Activity
1	15.084	Tetradecanoic acid	$C_{14}H_{28}O_2$	228.37	14.65		Fatty acid	Antioxidant, cancer preventive, nematicide, hypercholesterolemic, Lubricant
2	15.75	2,6,10-trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$	278	9.84		Olefins	Antiproliferative
3	16.20	Bicyclo[4.1.0]heptane, 7-butyl-	C_7H_{12}	96.170	2.3		Alkane	Activity not known
4	16.401	Decanoic acid, 8-methyl-methyl ester	$C_{10}H_{22}O_{11}$	172.26	3.86		Fatty acid	Flavor Nematicide Pesticide
5	16.96	1-undecanol	$C_{11}H_{24}O$	172.30	7.82		Fatty alcohol	Flavor, perfumery
6	17.15	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	24.61		Fatty acid	Antioxidant, hypocholesterolemicnematicide, hemolytic, 5-alpha reductase inhibitor
7	18.38	1-hexyl-1-nitrocyclohexane	$C_{12}H_{23}NO_2$	213.31	2.09		Ketone	Antioxidant, antimicrobial, anti-inflammatory
8	19.986	1,14-tetradecanediol	$C_{14}H_{30}O_2$	230.39	6.78		Alcoholic	Antimicrobial
9	20.148	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	$C_{18}H_{34}O_2$	282.46	19.18		Fatty acid	Hypocholesterolemic, antiarthritic, nematicide, 5-alpha reductase inhibitor, antiacne, hepatoprotective
10	21.619	2-hydroxy-3-[(9E) -9-octadecenoyloxy]propyl(9E)-9-octadecenoate	$C_{39}H_{72}O_5$	620.98	8.79		Ester	No activity reported

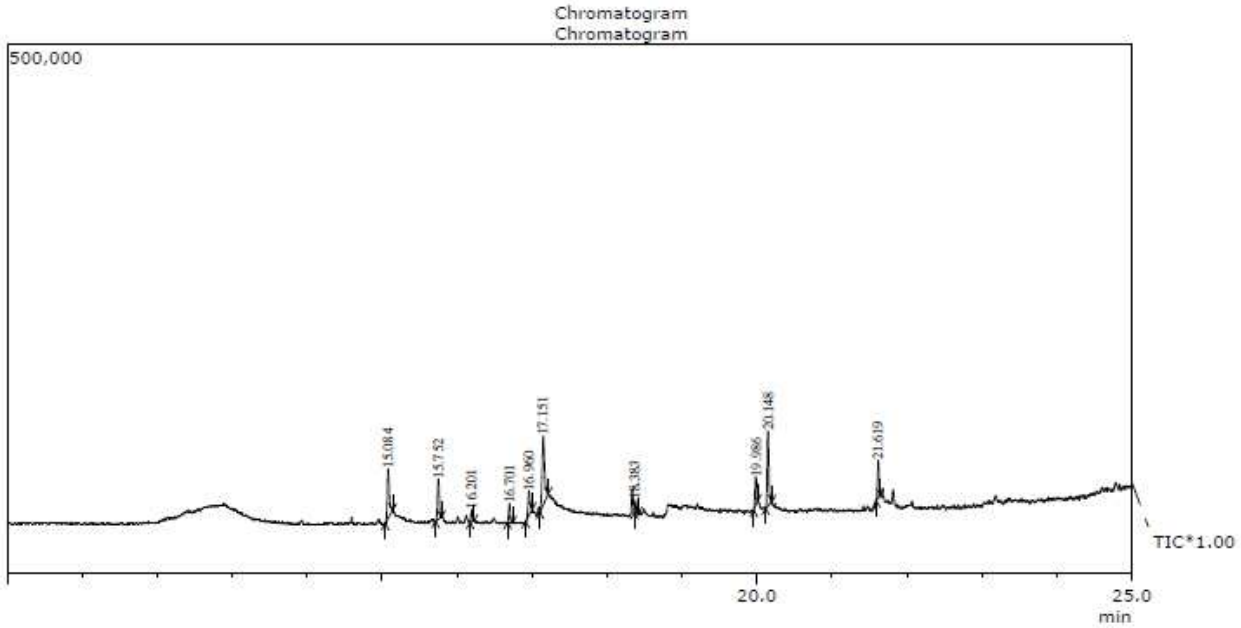
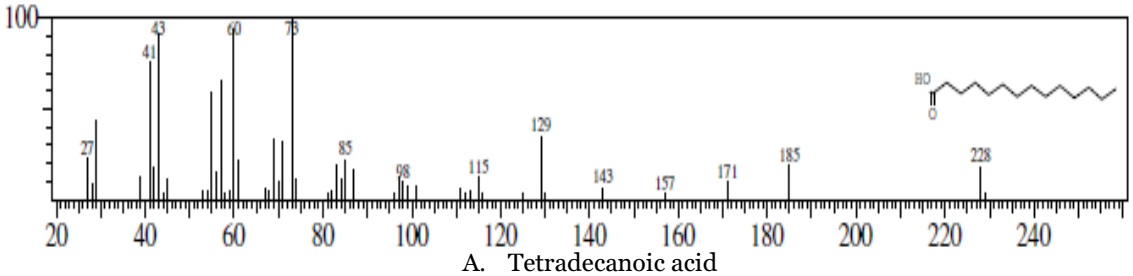
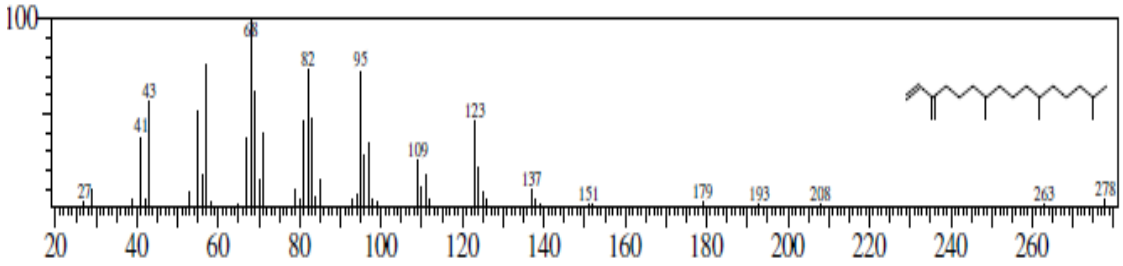


Fig 1: GC-MS Chromatogram of methanolic extract of *Eupatorium triplinerve*

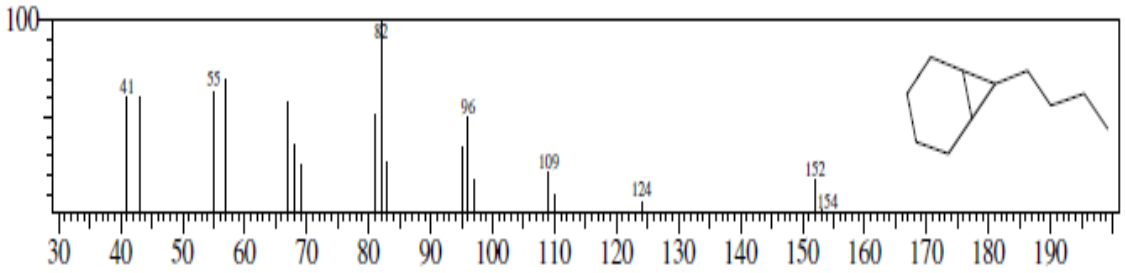
GC-MS analysis of *Eupatorium triplinerve* – Mass spectra



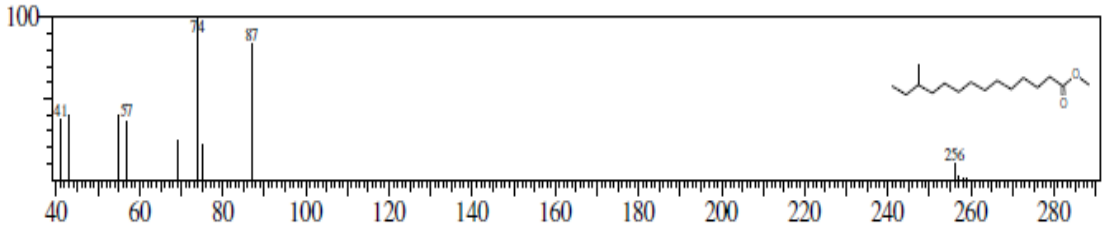
A. Tetradecanoic acid



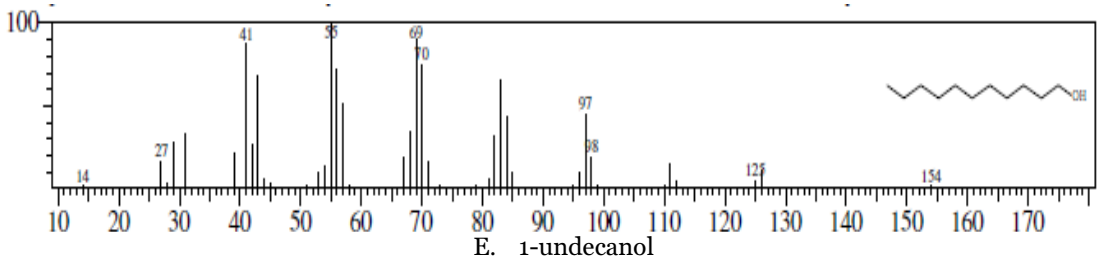
B. 2,6,10-trimethyl,14-ethylene-14-pentadecne



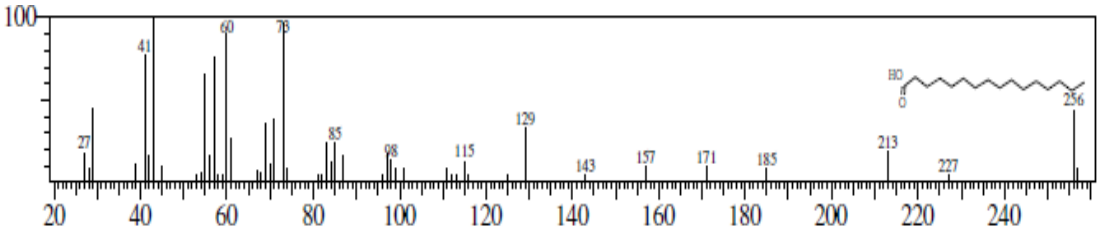
C. Bicyclo[4.1.0]heptane, 7-butyl-



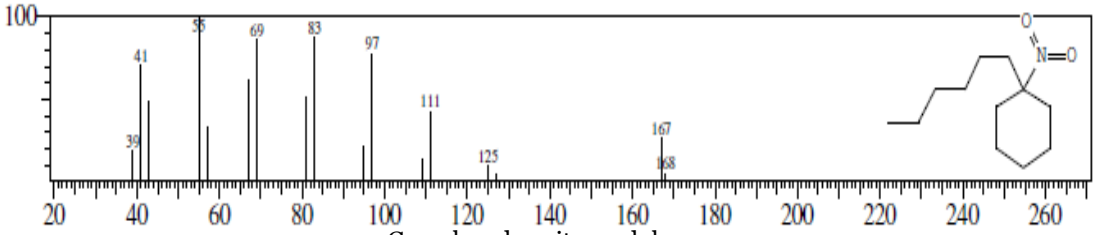
D. Decanoic acid, 8-methyl-, methyl ester



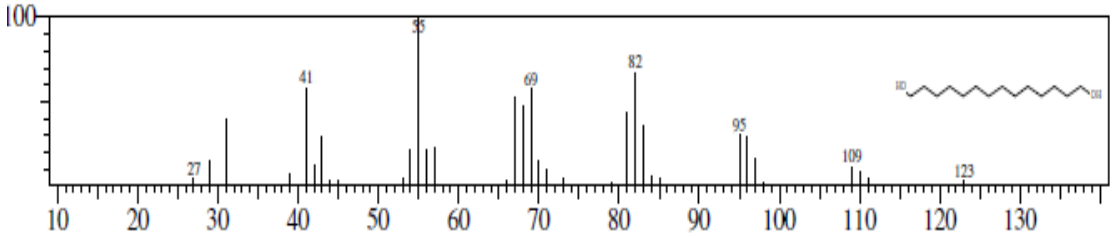
E. 1-undecanol



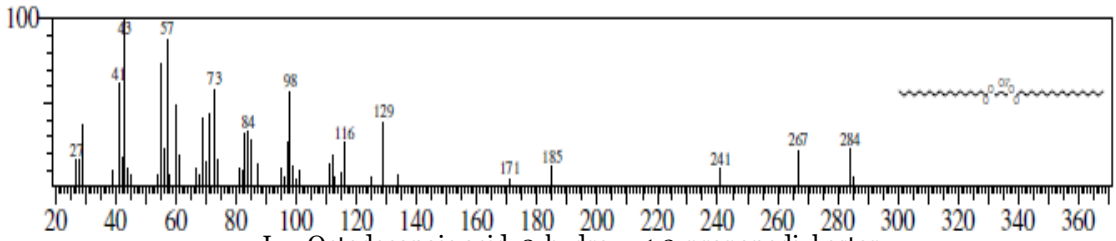
F. Hexadecanoic acid



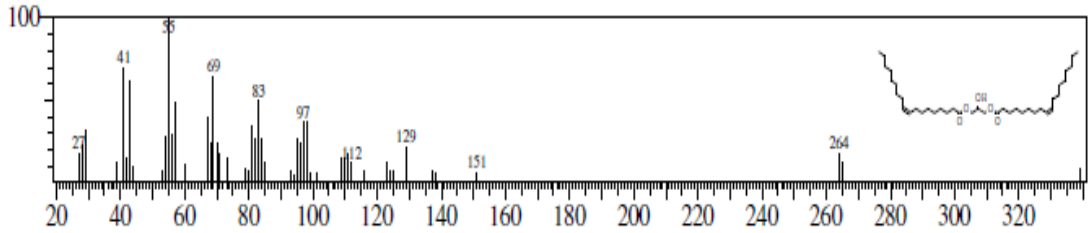
G. 1-hexyl-1-nitrocyclohexane



H. 1,14-tetradecanediol



I. Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester



J. 2-hydroxy-3-[(9E) -9-octadecenoyloxy]propyl(9E)-9-octadecenoate

Discussion

In the present study, the GC-MS analysis of the methanolic extract of *E triplinerve* showed the presence of ten compounds. In terms of percentage amounts hexadecanoic acid, tetradecanoic acid and octadecanoic acid were predominant in the extract. These three major compounds have all shown to have hypocholesterolemic activity, antioxidant and lubricating activity. Anticancer and antiproliferative are shown by tetradecanoic acid and 2,6,10,-trimethyl,14-ethylen-14-pentadecne, while 1-hexyl-1-nitrocyclohexane and 1,14-tetradecanediol other compounds show antimicrobial and anti-inflammatory activities.

There is growing awareness in correlating the phytochemical components and their biological activities [6,7,8]. *E triplinerve* is a plant used in Ayurvedic medicine however there are no reports on the thorough phytochemical analysis of the plant. We report the presence of some of the important components resolved by GC-MS analysis and their biological activities. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant and this type of study will be helpful for further detailed study.

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