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Research article

GAS CHROMATOGRAPHY–MASS SPECTROMETRY ANALYSIS OF CHLOROFORM EXTRACT OF *PHYLLANTHUS ACIDUS L.*

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ABSTRACT

The present study investigates the presence of phytochemical compounds in the chloroform extract of Phyllanthus acidus L, employed by Gas chromatography-Mass Spectrometry. 16 bioactive phytocompounds were found in the chloroform extract of Phyllanthus acidus. The prevailing compounds were (15.221%) of Z-5,17-octadecadien-1-ol acetate, followed by Tritetracontane (13.224%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol(11.730%), Octadecanoic acid, ethyl ester (7.962%), vitamin E (6.197%), Dotriacontane (4.118%) which has medicinal properties such as Antioxidant, Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide, Hypocholesterolemic, Nematicide, Pesticide, Lubricant. ,Antimicrobial, are used for the treatment of several ailments.

Keywords: *Phyllanthus acidus*, GC-MS, Leaf extract, Phytocompounds, Antioxidant.

INTRODUCTION

Use of plants as a source of medicine has been inherited and is an important component of the health care system [1]. Currently, medicinal plants are increasingly gaining importance in pharmaceutical and scientific societies, as they are the richest biological resources of traditional medicines, food supplements, and nutraceuticals . The official value of medicinal plants depends on their bioactive phytoconstituents. These phytochemicals attribute to the medicinal value of the plant, and that's why, plants are being used to cure several diseases all over the world since time immemorial. Screening of active compounds present in the plant leads to the invention of new drugs which are used to cure various serious diseases [2].

Medicinal plants are directly analyzed by *Gas chromatography-Mass spectrometry (GC-MS)* for their existing phytochemicals. GC-MS is an advanced technology to determine the presence of phytochemicals in plant tissues, which is extensively being utilized to screen the phytochemicals in medicinal plant species. Using GC-MS, it is now possible to identify volatile compounds with ease [3].

Phyllanthus acidus L skeels is a small tropical tree that grows up to 10m height, which bears pale yellow waxy fruits belonging to Euphorbiaceae family. It origins in Madagascar. The tree is now established as a native of the South American countries of Colombia and Brazil with wide distribution within tropical and subtropical countries also in south east Asia. It's leaves has been employed as one of the ingredients in a Thai remedy to control fevers, used as a dieting aid for people who are dieting and wish to remain slim. It has also been employed in the treatment of wide spectrum of diseases such an inflammatory, rheumatism, bronchitis, asthma, respiratory disorder, hepatic diseases and diabetes in India, Asia, the Caribbean region, and Central and south America [4].

The present study was aimed to investigate the possible chemical components by first preparing the Chloroform extract and separation and identification of the compounds by subjecting it to GC-MS analysis [5].

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MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL

Leaf of *Phyllanthus acidus* were collected from Maduravoyal,(2021) Chennai (Dt), Tamil Nadu, India.

PREPARATION OF LEAF POWDER

The leaves were collected and shade dried. It was powdered in mixer. The coarse powder sieved and was stored in a well closed container.

PREPARATION OF CHLOROFORM LEAF EXTRACT

Leaf Phyllanthus acidus were collected and authenticate, shade dried, coarsely powdered and extracted with chloroform by Maceration extraction. The powdered material is taken in a stoppered container with the chloroform and allowed to stand for at least 3-7 days in a warm place with frequent shaking. The mixture of powder containing solvent is filtered until most of the liquid drains off. The filtrate and washing are combined to produce 1000ml of the solution

GC-MS ANALYSIS

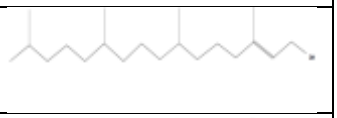
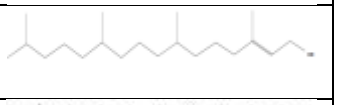
GC-MS analysis of chloroform extract of Phyllanthus acidus was performed using a Perkin-Elmer GC Clarus 600 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% biphenyl 95% dimethyl polysiloxane , 30m X 0.25mm ID X 250µm df) fused a capillary column [6].

For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy was operated in electron impact mode with ionization energy of 70Ev. Helium gas was used as carrier gas at a constant flow rate of 1ml/min and injection volume of 1µL was employed (a split ratio of 10:1). The injector temperature was set at 260ºc during the chromatographic reaction [7]. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60ºc (2min), followed by 300ºc at the rate of 10ºc min – 1 and 300ºc, where it was held for 6min. The solvent delay was 0 to 2min and the total GC-MS running time was 32min [8]. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in the analysis was Turbo-Mass Gold Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver 5.4.2 [9]. The spectrums of the component were compared with the database of spectrum of known components stored with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

RESULT

In the present study, a total of 16 different phytocomponents have been found in the Chloroform extract of the leaves of *Phyllanthus acidus*. The identified compounds of Phyllanthus acidus, their retention indices (RT), molecular formulae, molecular structure, molecular weight, and percentage composition (area%) are given in Table1.The results showed the presence of four major components with maximum percentage (15.221%) of Z-5,17-octadecadien-1-ol acetate, followed by Tritetracontane (13.224%), 3,7,11,15-tetramethyl-2-hexadecen-1-ol(11.730%) and Octadecanoic acid, ethyl ester (7.962%).The leaf extract also showed the presence of Dotriacontane (4.118%with RT of 27.11mins), Vitamin E (6.197% with RT of 26.70mins). The GC-MS Chromatogram also shows 16different peaks which confirm the presence of 16 compounds with their respective RT (Fig 1.) On comparison of mass spectra of each phytochemical with the NIST and Wiley library,16 phytoconstituents were characterized and identified. Some of the identified components possess biological activities which are listed in Table 2.

Table 1. Phytocomponents detected in the chloroform leaf extract of *Phyllanthus acidus*

S. No	Compound Name	% of Peak Area	Retention time (RT)	Molecular formula (MF)	Molecular weight (MW)	Compound structure
1.	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	11.730	18.06	$C_{20}H_{40}O$	296	
2.	3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	10.356	18.56	$C_{20}H_{40}O$	296	
3.	OCTADECANOIC ACID, ETHYL ESTER	7.962	19.64	$C_{20}H_{40}O_2$	312	
4.	1-OCTADECYNE	4.270	20.62	$C_{18}H_{34}$	250	

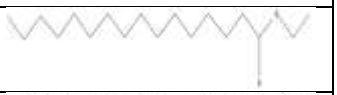
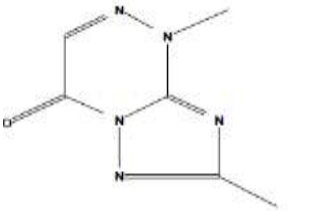
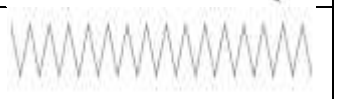
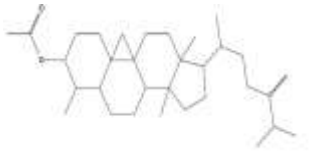
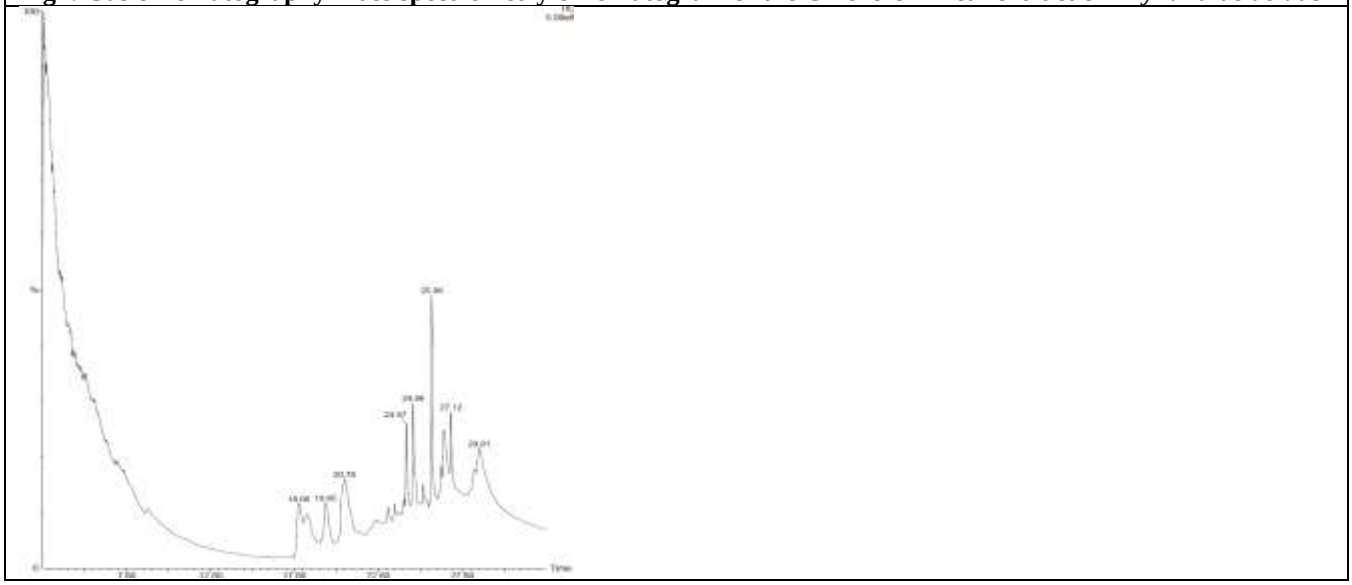
5.	Z-5,17-OCTADECADIEN-1-OL ACETATE	15.221	20.78	$C_{20}H_{36}O_2$	308	
6.	HEXADECANOIC ACID, ETHYL ESTER	1.286	23.36	$C_{18}H_{36}O_2$	284	
7.	DOTRIACONTANE	1.128	23.76	$C_{32}H_{66}$	450	
8.	2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL	6.243	24.45	$C_{15}H_{26}O$	222	
9.	SULFUROUS ACID, PENTADECYL 2-PROPYL ESTER	6.531	24.86	$C_{18}H_{38}O_3S$	334	
10.	TETRACOSANE, 1-BROMO	2.114	24.98	$C_{24}H_{49}Br$	416	
11.	1-CHLOROEICOSANE	1.404	25.46	$C_{20}H_{41}Cl$	316	
12.	TRITETRACONTANE	13.224	25.97	$C_{43}H_{88}$	604	
13.	**VITAMIN E**	6.197	26.70	$C_{29}H_{50}O_2$	430	
14.	**2,4-DIMETHYL-7-OXO-4,7-DIHYDRO-TRIAZOLO(3,2-C) TRIAZINE**	2.553	26.84	$C_6H_7ON_5$	165	
15.	**DOTRIACONTANE**	4.118	27.11	$C_{32}H_{66}$	450	
16.	**9,19-CYCLOERGOST-24(28)-EN-3-OL, 4,14-DIMETHYL-, ACETATE, (3. BETA., 4. ALPHA.,5. ALPHA.)-**	5.662	28.80	$C_{32}H_{52}O_2$	468	

Table 2. Nature of compound and Biological properties of some phytocomponents identified in the chloroform extracts of *Phyllanthus acidus* by GC-MS

Name of compounds	Nature of compound	Biological activity
3,7,11,15-TETRAMETHYL-2-HEXADECEN-1-OL	Terpene alcohol	Antimicrobial

HEXADECANOIC ACID, ETHYL ESTER	Fatty acid	Antioxidant, Hypo cholesterolemic, Nematicide, Pesticide, Lubricant, anti- androgenic, Flavor.
OCTADECANOIC ACID, ETHYL ESTER	Fatty ester	Hepatoprotective, antihistamine, hypochlolesterolemic, anti-eczemic, anti-oxidant, anticancer.
DOTRIACONTANE	_	Antimicrobial, anti-oxidant, anti-pasmodic.
2,6,10-DODECATRIEN-1-OL, 3,7,11-TRIMETHYL	Sesquiterpene alcohol	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide.
SULFUROUS ACID, PENTADECYL 2-PROPYL ESTER	_	No activity
TRITETRACONTANE	_	Anti-inflammatory, Antifungal, antibacterial.
VITAMIN E	Vitamin compound	Antitumor, Antispasmodic, Antioxidant, Vasodilator, Analgesic, Anti-diabetic, Anticancer, Antidermatitic.

Fig1: Gas chromatography-mass spectrometry chromatogram of the Chloroform leaf extract of *Phyllanthus acidus*



DICUSSION

GC-MS Chromatogram of the chloroform extract of the leaves of Phyllanthus acidus showed the presence of 16 different phytocomponents. From observations and comparison with NIST and Wiley library, it was found that Z-5,17-octadecadien-1-ol acetate, Tritetracontane, 3,7,11,15 tetramethyl-2-hexadecen-1-ol and Octadecanoic acid, ethyl ester are the major components(Fig.2). Besides these compounds, Dotriacontane (4.118%), Vitamin E (6.197%), and several other antioxidants, antimicrobial activities are also present. 3,7,11,15 tetramethyl-2-hexadecen-1-ol, which constitutes about 11.730%, is a Terpene alcohol which is used as an antimicrobial activity. However, Octadecanoic acid, ethyl ester which is 7.962%, is a Fatty ester which is used as an Hepatoprotective activity. Vitamin E 6.196%, is a Vitamin compound which is used as a maintain healthy skin and eyes, and strengthen the body's natural defence against illness and infection. Dotriacontane 4.118 can be used in the development of organogels.

CONCLUSION

In the presence study 16 chemical constituent have been found from chloroform extract of the leaves of *Phyllanthus acidus* by Gas chromatogram-Mass spectrometry analysis. The presence of various phytochemical contribute to the biological activity of the plant.

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