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GC–MS analysis of biologically active compounds in *Cayratia pedata* (lam) leaf and callus extracts

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In this study, the bioactive components of *Cayratia pedata* leaf and callus have been evaluated using GC/MS. The chemical compositions of the methanol extract of *Cayratia pedata* leaf and callus were investigated using Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extracts were matched with the National Institute of Standards and Technology (NIST) library. GC/MS analysis of methanolic extract of *Cayratia pedata* revealed the existence of twenty five compounds in leaf and twenty compounds in callus. The prevailing compounds of *Cayratia pedata* leaves were Hexadecanoic acid (28.46), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (30.62) and Hexadecanoic acid, methyl ester (27.94) whereas callus contains n-Hexadecanoic acid (13.67), Octadecanoic acid (17.04), Hexadecanoic acid, methyl ester (13.10) and 9,12-Octadecadienoic acid (16.05). The results of this study offer a platform of using of *Cayratia pedata* leaf and callus as herbal alternative for various diseases.

Keyword: *Cayratia pedata*, Callus, GC/MS, Bioactive components.

1. Introduction

India is called the botanical garden of the world for its rich natural resources. Over 6,000 plants in India are used in traditional, folklore and herbal medicine. The Indian system of medicine has identified 1500 medicinal plants of which 500 are commonly used [1]. Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function [2].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlorophyll etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from amino acids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [3]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [4]. The aim of this paper is to determine the organic compounds present in the *Cayratia pedata* leaves and callus extracts with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

2. Materials and Methods

2.1 Plant materials

The fully mature *Cayratia pedata* leaves were collected in April 2013 from Vandayar Iruppu, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Botanist, Dr. S John Britto, Department of Botany, St. Josephs College, Tiruchirappalli, Tamil Nadu, India. A Voucher specimen (SR 001) has been deposited at the Rapinat Herbarium, St. Joseph,s College, Tiruchirappalli, Tamil nadu, India.

2.2 Plant leaf Callus

The explants of Leaf from *Cayratia pedata* and washed with running tap water. It is then soaked in 2% Tween 20 solution for 5 min. After these washings, explants were taken out and dipped in 70% ethyl alcohol for 30 seconds. After alcohol dip, explants were surface sterilized with (0.1%) mercuric Chloride for 2 min followed by washing with distilled water 5 times for Surface sterilization. The sterilized explants were cultured on MS medium. The cultured callus was used for GC MS analysis.

2.3 Plant sample extraction

The collected *Cayratia pedata* leaves and callus were washed several times with distilled water to remove the traces of impurities from the leaves and callus. The leaves and callus were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extracts was obtained after complete elimination of alcohol under reduced pressure. The *Cayratia pedata* leaves and callus extracts were stored in refrigerator until used. Preliminary phytochemical tests were carried out on the ethanolic extract of *Cayratia pedata* leaves using standard procedures to identify the constituents as described by Sofowara [5], Trease and Evans [6] and Harborne [7, 8].

2.4 GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25 mm ID x 1 µMdf, composed of 100% Dimethyl polysiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI 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 ºC/min, to 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a TurboMass Ver 5.2.0.

2.5 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.

3. Results and Discussion

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions [9]. The phytochemical characters of the *Cayratia pedata* leaves and callus were investigated. The qualitative phytochemical analysis of ethanolic extract of *Cayratia pedata* leaves contains saponin, terpenoids, alkaloids, polyphenols and tannin. The qualitative phytochemical analysis of ethanolic extract of callus contains flavonoids, saponin, proteins, terpenoids, triterpenoids, alkaloids, polyphenols and tannin. Table 1 represents the phytochemical screening of *Cayratia pedata* leaves and callus extracts.

4. GC-MS Analysis

Twenty five compounds in *Cayratia pedata* leaf whereas twenty compounds in *Cayratia pedata* leaf callus were identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 2, 3 and Fig 1 and 2). The prevailing compounds *Cayratia pedata* leaves were Hexadecanoic acid (28.46), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl (30.62) and Hexadecanoic acid, methyl ester (27.94) whereas callus contains n-Hexadecanoic acid (13.67), Octadecanoic acid (17.04), Hexadecanoic acid, methyl ester (13.10) and 9,12-Octadecadienoic acid (16.05). Table 4 represents the biological activity of phyto-compounds.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases.

Table 1: Shows phytochemical screening of *Cayratia pedata* leaves and callus extracts

S. No	Phytochemicals	Results	
		Leaf	Callus
1	Tannin	+	++
2	Phlobatannins	-	-
3	Saponin	+	+
4	Flavonoids	+	+
5	Steroids	-	-
6	Terpenoids	+	+
7	Triterpenoids	+	+
8	Alkaloids	+	+
9	Carbohydrate	+	++
10	Protein	--	++
11	Anthroquinone	-	-
12	Polyphenol	+	++
13	Glycoside	-	-

(-) Absent (+) Present (++ = High concentrations)

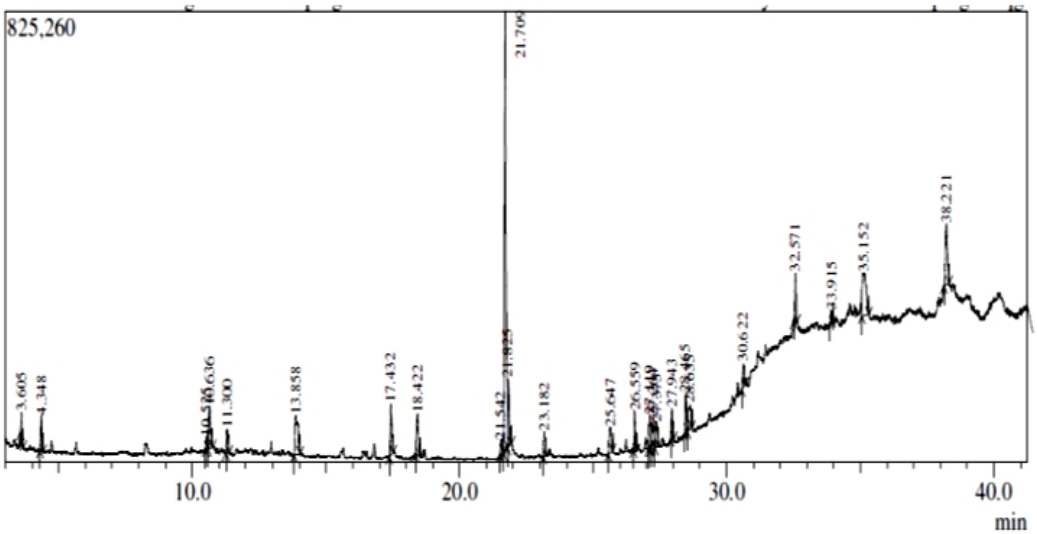

Fig 1: shows Determination of phyto-contitutents of *Cayratia pedata* leaf by GC MS

Table 2: Shows Determination of phyto-contitutents of *Cayratia pedata* leaf by GC MS

Peak	R. Time	Area %	Name of the compound	Molecular formula	Molecular weight
1.	3.605	1.14	Butanoic acid, 2-methyl-, methyl ester	$C_6 H_{12} O_2$	116
2.	4.348	1.59	Pentanoic acid, methyl ester	$C_6 H_{12} O_2$	116
3.	10.525	0.74	3-Cyclohexen-1-ol, 4-methyl-1-(1-methyleth	$C_{10} H_{18} O$	154
4.	10.636	3.78	1H-Indene, 1-methylene-	$C_{10} H_{18}$	128
5.	11.300	1.60	Decanal (CAS) n-Decanal	$C_{10} H_{20} O$	156
6.	13.858	5.14	Cyclohexasiloxane, dodecamethyl		
7.	17.432	3.69	1,2-Benzenedicarboxylic acid, DI	$C_{10}H_{10}O_4$	194
8.	18.422	3.73	Cycloheptasiloxane,	$C_{14}H_{42}O_7Si_7$	518
9.	21.542	0.94	1-Isopropenyl-3-Propenyl-Cyclop	$C_{11}H_{18}$	150
10.	21.709	33.24	1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12} H_{14} O_4$	222
11.	21.825	4.69	1,2-Benzenedicarboxylic acid, diethyl ester	$C_{12} H_{14} O_4$	222
12.	23.182	1.54	2,6-dihydroxybenzoic acid 3TMS	$C_{16} H_{30} O_4 Si_3$	370
13.	25.647	2.62	1,3-Diphenyl-1,3,5,5-Tetramethyl-C	$C_{16}H_{22}O_3Si_3$	346
14.	26.559	1.93	2,6,10-Trimethyl,14-Ethylene-14-PE	$C_{20}H_{38}$	278
15.	27.119	1.90	1,2-Benzenedicarboxylic acid, bis(2-methylp	$C_{16} H_{22} O_4$	278
16.	27.264	3.79	trans-p-Mentha-1(7),8-dien-2-ol	$C_{10} H_{16} O$	152
17.	27.375	2.21	Phosphine oxide, BIS(Pentamethy	$C_{22} H_{31} O P$	342
18.	27.943	1.45	Hexadecanoic acid, methyl ester (CAS) Meth	$C_{17} H_{34} O_2$	270
19.	28.465	2.54	Hexadecanoic acid $$ Hexadecan	$C_{16}H_{32}O_2$	256
20.	28.635	3.21	1,2-Benzenedicarboxylic acid, dibutyl ester	$C_{16} H_{22} O_4$	278
21.	30.622	1.28	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R	$C_{20} H_{40} O$	296
22.	32.571	2.72	4-p-chorophenyl-2-dimethylamino-5-nitrosot	$C_{12} H_{13} N_3 O S$	247
23.	33.915	0.63	Dimethyl(phenyl)(3-[(trimethyls	$C_{14}H_{26}OSi_2$	266
24.	35.152	7.57	2,6,10,14-Hexadecatetraensaeure	$C_{22}H_{36}O_2$	332
25.	38.221	6.34	1,2-Benzenedicarboxylic acid, bis(2-ethylhex	$C_{24} H_{38} O_4$	390

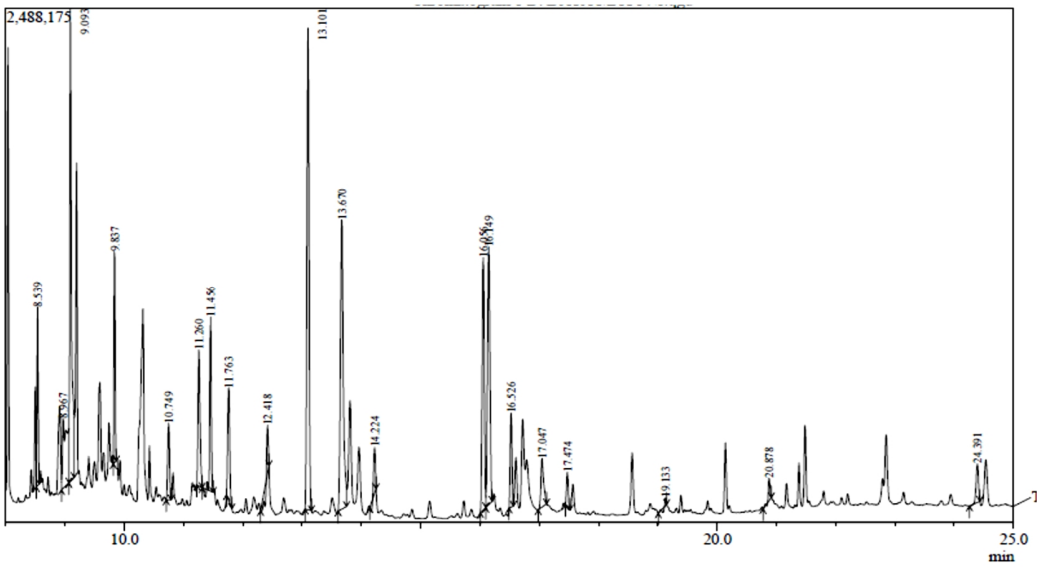

Fig 2: Shows Determination of phyto-contitutents of *Cayratia pedata* leaf Callus by GC MS

Table 3: shows Determination of phytoconstituents of *Cayratia pedata* leaf Callus by GC MS

Peak	R. Time	Area %	Name of the compound	Molecular formula	Molecular weight
1	8.539	3.53	1-Hexadecene	$C_{16}H_{32}$	224
2	8.967	5.28	Cyclohexane, undecyl-	$C_{17}H_{34}$	238
3	9.093	13.68	3-Hexadecene, (z)-	$C_{16}H_{32}$	224
4	9.837	4.28	1-Heptadecene	$C_{17}H_{34}$	238
5	10.749	2.31	Homogentisic acid, bis(tert-butyldimeth	$C_{26}H_{50}O_4Si_3$	510
6	11.260	4.47	Phenazine	$C_{12}H_8N_2$	180
7	11.456	4.04	n-Pentadecanol	$C_{15}H_{32}O$	228
8	11.763	3.68	n-Nonadecanol-1	$C_{19}H_{40}O$	284
9	12.418	0.47	8-Octadecanone	$C_{18}H_{36}O$	268
10	13.101	16.39	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270
11	13.670	13.54	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
12	14.224	1.15	Behenic alcohol	$C_{22}H_{46}O$	326
13	16.056	8.85	9,12-Octadecadienoic acid (z,z	$C_{19}H_{34}O_2$	294
14	16.149	10.22	9-Octadecenoic acid (Z)-, meth	$C_{19}H_{36}O_2$	296
15	16.526	2.80	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298
16	17.047	2.17	Octadecanoic acid	$C_{18}H_{36}O_2$	284
17	17.474	0.96	9-Tricosene, (Z)-	$C_{23}H_{46}$	322
18	19.133	0.06	Octadecanoic acid, methyl		
19	20.878	0.60	4-Hydroxyphenylacetic acid, ethyl ester	$C_{16}H_{26}O3Si$	294
20	24.391	1.51	2,6,10,14,18,22-Tetracosahexaene, 2,6,	$C_{30}H_{50}$	410

Table 4: Biological Activity of phyto-components identified in the methanolic extract of the *Cayratia pedata* leaf and callus by GC-MS.

Sl. No.	Name of the Compounds	Biological activity**
1	n-Hexadecanoic acid (Leaf and callus)	Antioxidant, hypocholesterolemic, nematicide, pesticide, anti-androgenic flavor, hemolytic and 5-Alpha reductase inhibitor
2	1,2-Benzenedicarboxylic acid, diisooctyl ester **(Callus)**	Used as Softeners, Used in preparation of perfumes and cosmetics, Used as plasticized vinyl seats on furniture and in cars, and clothing including jackets, raincoats and boots. Used in textiles, as dyestuffs, cosmetics and glass making.
3	Hexadecanoic acid, methyl ester **(Callus)**	Antioxidant Hypocholesterolemic Nematicide Insecticide Lubricant Antiandrogenic Flavor, Hemolytic

****Source:** Dr. Duke's phytochemical and ethnobotanical databases [Online database].

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