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GC-MS ANALYSIS OF PHYTOCHEMICALS IN THE METHANOLIC EXTRACT OF *CYCLEA PELTATA. (*LAM) HOOK.F & THOMSON

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ABSTRACT

Cyclea peltata is locally called as “kariballi” belongs to the family Menispermaceae. It is a twining shrub, traditionally used in the treatment of wounds, skin infections and muscular sprains by local folk medicinal practioners. The present study was designed to determine the phytochemical compounds in the methanolic extract of *Cyclea peltata* by GC-MS analysis. GC-MS chromatogram of the methanolic extract showed six major peaks at retention time of 31.278, 32.742, 35.713, 35.881, 41.244 and 48.947 minutes. The spectrum of the unknown components in the methanol extract was compared with the spectrum of known compounds stored in the NIST library. The mass of the compounds and fragments recorded were matched with NIST database for identification of probable compounds present in the sample and from GC-MS results Twenty five phytochemical compounds were identified.

KEYWORDS: **GC-MS analysis, phytochemicals, *Cyclea peltata*, methanolic extract**

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INTRODUCTION

Cyclea peltata is a member of the family Menispermaceae, in ayurveda it is commonly known as *Rajpatha* [1]and its local name is kariballi. It is a slender, much branched twining shrub found throughout South and East India.Roots are tuberous and are traditionally used in the treatment of cough, urinary disorder, snake poisoning, jaundice, diabetes, fever, stomach ache and asthma[2,3]. Leaves are peltate, hairy and are two to three centimeters long. Leaves are traditionally used as coolant, antidandruff and diuretic. Flowers are very small, male flowers are arranged in long panicles. Fruits are drupe and reniform. The species of this plant are easily distinguished by the cup-shaped calyx and corolla[4].Traditionally local folk medicine practioners residing in Nagapura haadi and Haemmegae haadi tribal settlements of Hunsur taluk, Mysuru district, use this plant as one of the ingredients in their herbal formulations for treatment of wounds, skin infections and muscular sprains. Recent studies have shown that *Cyclea peltata* possesses anti oxidant activity[5], anti-diuretic activity[3], hepatoprotective activity[6], anti-hyperlipidemic activity[7] and anti-diabetic activity[8].It has been reported that tuberous roots of *Cyclea peltata* have capability of treating Nephrolithiasis[9] and Type 2 diabetes[10]. It contains tetrandrine and a bisbenzylisoquinoline dioxine having antioxidant activity[11]. Leaves contain alkaloids such as cycleanine, bebeerines, hayatinin, hayatidin and hayatin which are having medicinal value[3].There has been not much information available on phytochemical components in the methanolic extract of the entire aerial part of *Cyclea peltata,* hence the present study was designed to identify phytochemical compounds in the methanolic extract of *Cyclea peltata* by GC-MS analysis.

MATERIALS AND METHODS

(i) Authentication of plant material

Plant materials were collected from Nagapura haadi and Haemmegae haadi tribal settlements of Hunsur taluk, Mysuru district, Karnataka, India. The plants were photographed, taxonomically identified and their herbarium specimens were made. Herbarium specimens were deposited in the Department of Pharmacognosy, Herbarium Collection Centre, JSS College of Pharmacy, Mysuru, Karnataka, India.

(ii) Collection of plant materials

Fresh plant materials (*entire aerial part of Cyclea peltata* comprising only leaves and stem) were collected and were washed in sterile distilled water and cleaned plant material were wiped with clean dry cloth. The plant material was shade dried under room temperature for seven days and was ground into coarse powder.

(iii) Extraction of Plant Material

Twenty grams of powdered sample was subjected to soxhlet extraction using 100mL of Methanol for eight hours at 50^{o}C. The methanolic extract was filtered through Whatmann No. 1 filter paper and filtrate was evaporated in rotary evaporator, dried in desiccators and stored until further use.

(iv) Preparation of stock solution

The extract was reconstituted in methanol. 1 µl of the methanolic extract was employed in GC-MS for the analysis of different compounds.

(v)Gas Chromatography-Mass Spectrometry analysis [12]

GC-MS analysis of the methanol extract of *Cyclea peltata* (*aerial part*) was performed using a GC- MS Clarus 500 Perkin Elmer system which comprised a AOC- 20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument. GC was equipped with a fused capillary column Restek Rtx^{R} – 5, (30meter X 0.25 mm) (5% diphenyl / 95% dimethyl polysiloxane), running in electron impact mode at 70 eV. For GC-MS detection, an electron ionization system was operated in an electron impact mode with ionization energy of 70 eV. Helium (99. 999%) was used as carrier gas at a constant flow rate of 1ml/min.1.0 µl volume of methanolic extract of *Cyclea peltata* (aerial part) was injected to GC-MS which splits the components into 10:1 ratio and the injector temperature was maintained at 280^{0}C, the ion-source temperature was 200°C. The oven temperature was programmed from 40^{0}C

(isothermal for 5 min.), with an increase of 6^0C/ min to 280^0C, then ending with a fifteen minutes isothermal at 280°C. Mass spectra were taken at 70 eV; a 0.5 seconds of scan interval and fragments from 40 to 550 Da. The total GC running time was 60 minutes.The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

(vi)Identification of phytochemical components

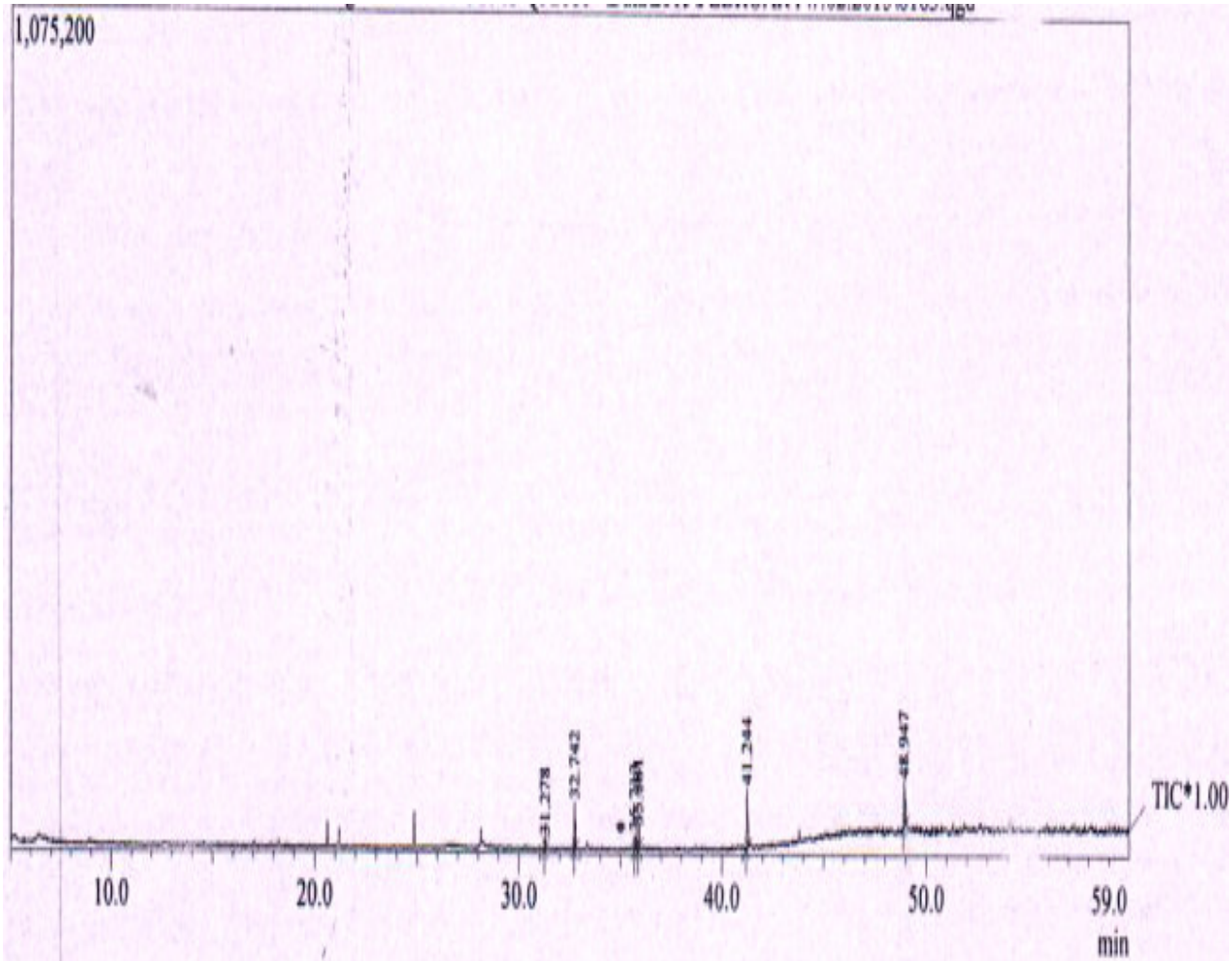
National Institute Standard and Technology (NIST) database having more than 62,000 patterns was used for interpretation on mass-spectrum GC-MS obtained for the respective plant extract. The spectrum of the unknown compounds were compared with the spectrum of known components stored in the NIST library and the name, molecular weight, and structure of the components of the test plant extract was ascertained.

RESULTS AND DISCUSSION

GC-MS chromatogram of the methanolic extract of *Cyclea peltata* aerial part showed six major peaks at retention time of 31.278, 32.742, 35.713, 35.881, 41.244 and 48.947 minutes (Figure-1) and extracted ion chromatograms were obtained from matching all these major peaks with NIST library. The mass of the compounds and fragments recorded were matched with NIST database for identification of probable compounds present in the sample. Twenty five phytochemical compounds were identified in the methanolic extract of the aerial part of *Cyclea peltata* by GC-MS. Names of these phytocompounds along with its molecular formula and molecular weight details are all listed in table 1. From the results, it was observed that Palmitic acid, beta-monoglyceride, Glycerol 1-monopalmitate, 2,3-Dihydroxypropyl hexadecanoate, 1,2-Dipalmitin, Pentadecanoic acid were found to be the major components constituting about 30.59% of the extract. The second major components in the extract were Hexadecyl trichloroacetate, [(Dodecyloxy)methyl]oxirane, 3-Eicosene, 2-methyl-1-hexadecanol, Vinyl octadecyl ether constituting about 29.23 % of the extract. Methyl hexadecanoate, Methyl tridecanoate and Methyl caprate are the third major components in the extract constituting about 20.60 % of the extract. Whereas phytocomponents such as 1-Nitrohexane, 3-Methylbutanenitrile, 4-Penten-1-yl acetate and Imidazole-1-carboxylic acid isopropyl ester were found to be in a very less quantity constituting about 4.48 % of the extract. The percentage of all the phytocompounds present in the extract were all listed in the table 1.

Figure 1

GC-MS Chromatogram of the aerial part methanolic extract of Cyclea peltata



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Height	Height%	A/H	Mark	Name
1	31.278	31.242	31.317	30945	4.48	14690	5.69	2.10	MI	
2	32.742	32.700	32.792	142370	20.60	61039	23.66	2.33	MI	
3	35.713	35.683	35.750	44022	6.37	20134	7.80	2.18	MI	
4	35.881	35.850	35.925	60315	8.73	27209	10.55	2.21	MI	
5	41.244	41.200	41.308	211390	30.59	77960	30.22	2.71	MI	
6	48.947	48.892	49.008	201943	29.23	56978	22.08	3.54	MI	
				690985	100.00	258010	100.00			

Table 1

Phytochemical constituents identified in the aerial part methanolic extract of Cyclea peltata by GC-MS along with its molecular formula, molecular weight details.

Retention time (min)	Name of the Compounds	Molecular Formula	Molecular weight	% Area
31.275	1-Nitrohexane	$C_6H_{13}NO_2$	131.17	4.48
	3-Methylbutanenitrile	C_5H_9N	83.1317	
	4-Penten-1-yl acetate	$C_7H_{12}O_2$	128.16	
	Imidazole-1-carboxylic acid isopropyl ester	$C_7\ H_{10}\ N_2\ O_2$	154.16	
32.742	Methyl hexadecanoate	$C_{17}H_{34}O_2$	270.45	20.60
	Methyl tridecanoate	$C_{14}H_{28}O_2$	228.37	
	Methyl caprate	$C_{11}H_{22}O_2$	186.29	
35.713	Trans-2-hexenyl butyrate	$C_{10}H_{18}O_2$	170.24	6.37
	4,4-Dimethyl-1-hexene	C_8H_{16}	112.21	
	2-Bromo-6-methylheptane	$C_8H_{17}Br$	193.12	
	1-Bromo-2-methylbutane	$C_5H_{11}Br$	151.04	
35.881	Methyl stearate	$C_{19}H_{38}O_2$	298.50	8.73
	Methyl tridecanoate	$C_{14}H_{28}O_2$	228.37	
	Methyl myristate	$C_{15}H_{30}O_2$	242.39	
	Hexadecanoic acid	$C_{18}H_{36}O_2$	284.47	
41.244	Palmitic acid, beta-monoglyceride, Glycerol 1-monopalmitate 2,3-Dihydroxypropyl hexadecanoate	$C_{19}H_{38}O_4$	330.50	30.59
	1,2-Dipalmitin	$C_{35}H_{68}O_5$	568.00	
	Pentadecanoic acid	$C_{18}H_{36}O_4$	316.00	
48.947	Hexadecyl trichloroacetate	$C_{18}H_{33}Cl_3O_2$	387.81	29.23
	[(Dodecyloxy)methyl]oxirane	$C_{15}H_{30}O_2$	242.39	
	3-Eicosene	$C_{20}H_{40}$	280.53	
	2-methyl-1-hexadecanol	$C_{17}H_{36}O$	256.46	
	Vinyl octadecyl ether	$C_{20}H_{40}O$	296.53	

***Source: NIST Standard Reference Database Number 05*

CONCLUSION

Cyclea peltata was used by folk medicinal practioners for treating various ailments since from very long time for many generations and there has been not much information available on phytochemical components of this plant extracts. With this background among the twenty five phytochemical compounds identified in the present study a few of them may have some useful therapeutic activity which may be of pharmacological importance. Hence further isolation of these compounds and their screening for specific bioactivity is required for developing novel therapeutic agents for treating different ailments.

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