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Sujesh M
Asso. Professor, Dept. Pharmaceutical Sciences, CPAS, Puthuppally Campus, Kottayam, Kerala, India

Dr. Jyoti Harindran
Principal and Head of Research Department, Dept. Pharmaceutical Sciences, CPAS, Cheruvandoor Campus, Kottayam, Kerala, India

Correspondence
Sujesh M
Asso. Professor, Dept. Pharmaceutical Sciences, CPAS, Puthuppally Campus, Kottayam, Kerala, India

Phytochemical analysis and GC-MS profiling of the various extract of *Cyclea peltata* Root (Hooks and Thom.)

Sujesh M and Dr. Jyoti Harindran

Abstract
Plants have been an important source of medicine since the beginning of human civilization and it still continues as one of the major source of drugs in modern as well as traditional medicine throughout the world. Despite the major advances in the modern medicine, the development of new drug from natural products is still considered important. Cyclea peltata of Menispermaceae family is commonly known as "Patha" and is herb which in mentioned in all ancient scriptures of Ayurveda. The great sage Charaka has categorized patha as "Sadhaniya"-means a healing herb. The roots have great medicinal value and are used both internally and externally. Externally it is used to treat infected wounds, sinuses, skin diseases and pruritus. Internally it is used as an anti-inflammatory, gastro-protective, anti-oxidant, immuno-modulatory, antiseptic etc. The present study deals with the qualitative and quantitative phytochemical analysis and GC-MS profiling of the ethyl acetate (CPEA) and petroleum ether extract (CPPE) of the root of Cyclea peltata. Phytochemical analysis shows the presence alkaloids, steroid, terpenoid, tannins in ethyl acetate extract (CPEA) and alkaloid and saponins in pet. ether extract (CPPE). The GC-MS chromatogram shows the presence of hexadecanoic acid, ethyl-9-octadecenoate for ethyl acetate extract (CPEA) and n-decanoic acid and hexadecanoic acid, cis-9-hexadecinal etc. for pet. ether extract (CPPE).

Keywords: Cyclea peltata root, phytochemical analysis, GC-MS profile, ethyl acetate extract

1. Introduction
Medicinal plants are the "backbone" of traditional medicine, which means more than 3.3 billion people in the less developed countries utilize medicinal plants on a regular basis [1]. Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis [2]. It has provided a complete store house of remedies to cure all ailments of mankind. Plants have been major source of medicine in all cultures from ancient times [3]. In the traditional system, various indigenous plants are being used in the diagnosis, prevention and elimination of physical, mental or social imbalance. Despite the major advances in the modern medicine, the development of new drugs from natural product is still considered important.

Cyclea peltata is a slender twining shrub, climbing in tall trees and belonging to the Menispermaceae family. It is grown throughout India and Srilanka. It is commonly known as "Patha" or "Rajpatha" [4]. The Kani and Kurichiya tribal people of Kannur district in Kerala has been cultivating this for generation for its medicinal properties [5]. It flowers and fruits in the month April-May. It is seen in the evergreen and semi-evergreen forest and distributed in Andaman and Nicobar Islands, Arunachal Pradesh, Assam, Maharashtra etc., [6].

The roots of patha have great medicinal value and are used for medicinal purpose, both, internally as well as externally. External application of the paste of its roots and leaves is extremely beneficial, in infected wounds, sinuses, and skin diseases like erysipelas and pruritus. The external application of this paste is said to be useful in serpent bite also. Internally, patha keen stimulant for digestive system and endows the actions like appetizer, digestant, astringent, and vermicide. The roots are also used in urinary ailments like dysuria and cystitis and as blood purifier [7]. The leaves of Cyclea peltata are found to contain alkaloids such as cycleanine, berberine, hayatinin, hayatidin and hayatin. Root contains bis benzyl isoquinoline alkaloids, cycleapeltine, cycleadrine, cycleacuine, cycleanorine and cycleahomine chloride [8].

2. Methodology

2.1 The plant material and extraction

The root plant was collected from Mavoor village, Kozhikode, Kerala. It was cleaned, shade dried, powdered and extracted by maceration with petroleum ether and ethyl acetate separately. It was authenticated by Dr. M.V. Krishnaraj of Baselius College Kottayam.

2.2 Preliminary phytochemical screening

The test plant extracts was subjected to preliminary qualitative phytochemical screening as per the standard procedures to determine the presence of various phytoconstituents such as tannins, alkaloids, saponins, flavanoids, phytosterols, and triterpenoids in the extracts [9].

2.3 Quantitative estimation of Alkaloid

The quantitative estimation of alkaloids in ethyl acetate (CPEA) and pet. ether (CPPE) was performed by spectrophotometric method of Dragendorff's reagent [10].

10 mg of the each extract was accurately weighed and made upto 1 mL with DMSO. The sample was centrifuged over 10 min (3000 rpm) to remove residual suspended particles and then 0.5 mL extracts were mixed with 1 mL of HCl (0.1 N). Then 0.25 mL of Dragendorff's reagent was added to the previous mixture for precipitation and the precipitate were centrifuged over 5 min (3000 rpm). This precipitate was further washed with 0.25 mL of ethanol. The filtrate was discarded and the residue was then treated with 0.25 mL of disodium solution (1% w/v). The brownish black precipitate formed was then centrifuged for 5 min at 3000 rpm. This residue was dissolved in 0.2 mL of concentrated nitric acid and 0.1 mL was then pipetted out and mixed with 0.5 mL of thiourea solution (3% w/v). The absorbance of this solution was measured at 435 nm against a blank containing 0.1 mL of concentrated nitric acid and 0.25 mL of thiourea solution (3% w/v).

2.4 GC-MS profiling

The two extracts were subjected to GC-MS studies. The instrument was Shimadzu GC-MS model no. QP 2010 S equipped with an Rxi-5 Sil MS capillary column 30 m length, 0.25 mm internal diameter, 0.25µm film thickness. The column oven temperature was 80 ^{0}C for 2 minutes and the temperature was gradually increased to 280 ^{0}C at 5 ^{0}C per minute for sample CPPE and and 60^0 C gradually increased to 260 ^{0}C at 5 ^{0}C per minute for sample CPEA. 1 microliter sample was injected for analysis. Helium gas 99.9% was used as carrier gas was 3 mL/min. Sample injected temperature was maintained at 280 ^{0}C for CPPE and 260 ^{0}C for CPEA and split ratio was 50 for both samples throughout the experiment period. The ionization mass was done at elution impact mode 70 eV. The mass spectrum was recorded for the mass range 50-500 m/z for CPPE and 50-650 m/z for CPEA and was scanned at a rate of 0.5 mL/sec. The total running time for GC-MS was 45 minutes. The compound separated on elution through column was detected in electronic signal. The m/z ratio obtained was calibrated through graph obtained which was called as mass spectrum which is the finger print of the molecule. The identification of the compound was done by GCMS software called GCMS solutions, based on the comparison of their mass spectra with NIST 11 and WILEY-8 Libraries. The relative percentage of each extract constituent was expressed as percentage with peak area normalisation.

3. Result and Discussion

The pet. ether extract of Cyclea peltata (CPPE) shows the presence of alkaloid, and saponin. The ethyl acetate extract of Cyclea peltata (CPEA) shows the presence of alkaloid, steroids, terpenoids and tannins.

Table 1: Shows the presence/absence of phytoconstituents in CPEA and CPPE extract of Cyclea peltata after preliminary phytochemical screening.

Figure	Phytoconstituents	CPEA	CPPE
1	Alkaloids	+	++
2	Flavanoids	-	-
3	Glycosides	-	-
4	Saponins	-	+
5	Terpenoids	+	-
6	Steroids	+	-
7	Phenols	-	-
8	Tannins	+	-

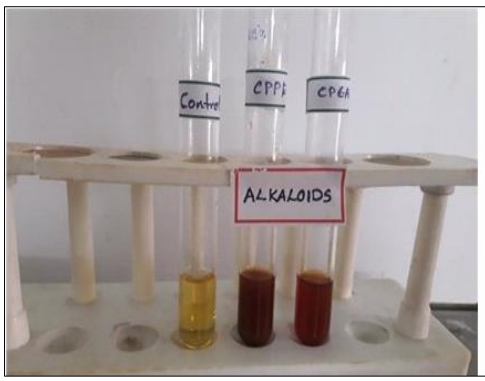

Fig 1: Alkaloids

Fig 2: flavonoids

Fig 3: Glycosides

Fig 4: Saponins

Fig 5: terpenoids

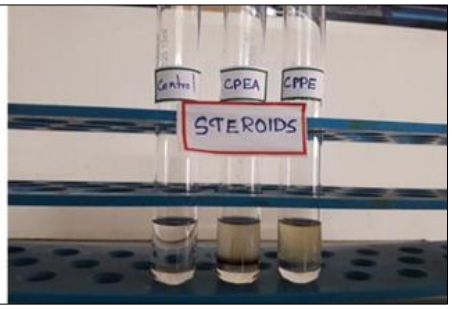

Fig 6: Steroids

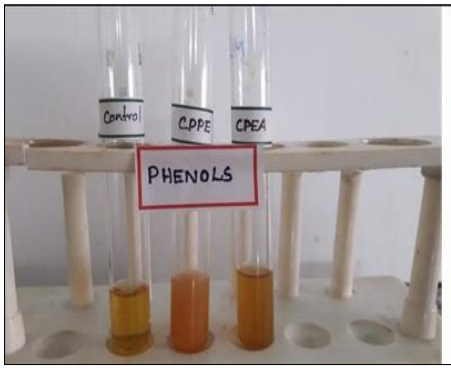

Fig 7: Phenols

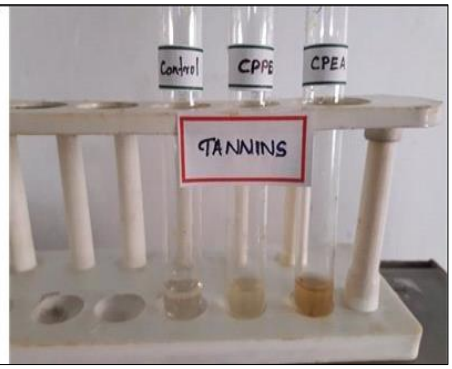

Fig 8: Tennins

Quantitative analysis of CPEA extract and CPPE extract using alkaloid ajmaline as standard shows that CPEA extract have 0.0026mg/mg of sample and CPPE extract have 0.0085mg/mg of the sample respectively in terms of ajmaline units (Fig. 9 and Table: 2 and 3).

Table 2: Ajmaline standard

Concentration(µg/mL)	Absorbance
100	0.1467
200	0.2246
400	0.4092
800	0.7789
1000	0.9554

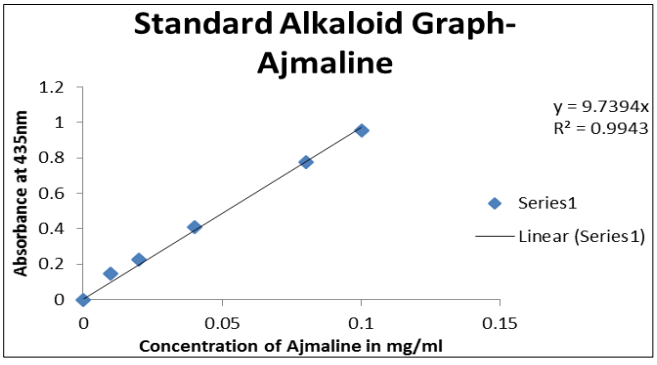

Fig 9: Standard alkaloid graph of alkaloid ajmaline

Table 3: shows the concentration of alkaloid present in CPEA and CPPE extract of root of Cyclea peltata in terms of ajmaline presence /absence of phytoconstituents in CPEA and CPPE extract of Cyclea peltata after preliminary phytochemical screening.

Sample code	Absorbance at 435nm	Amount of Alkaloids in terms of Ajmaline units(mg/mg)
CPEA	0.1282	0.002633
CPPA	0.4137	0.008495

GCMS profiling of CPEA showed major peaks at retention time of 29.956, 33.213, 33.648, 36.697, and 37.034 (fig.10). The Pet. Ether extract (CPPE) showed major peaks at retention timeof 19.600, 26.908, 27.272, 28.039 snd 31.809 (fig. 11). The major peaks obtained were compaired with the NIST 11 and WILEY-8 Libraries.

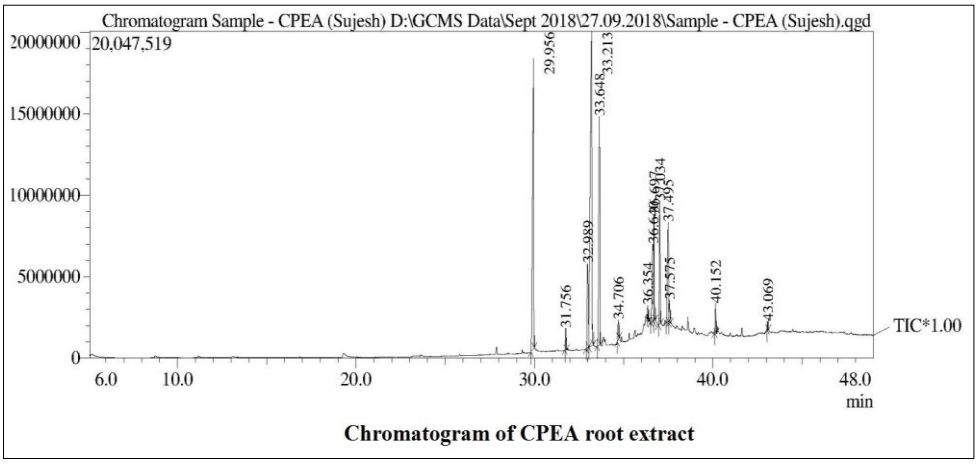

Fig 10: Chromatogram of CPEA extract

Table 4: shows GC-MS profile CPEA extract

Peak Report TIC							
Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	29.956	85727609	22.07	17819383	20.17	HEXADECANOIC ACID, ETHYL ESTER	88.10
2	31.756	3483920	0.90	1337596	1.51	HEPTADECANOIC ACID, ETHYL ESTER	88.10
3	32.989	21613529	5.56	5189974	5.88	ETHYL LINOLATE	67.10
4	33.213	123288506	31.74	19298718	21.85	ETHYL 9-OCTADECENOATE	55.10
5	33.648	53745749	13.84	14046334	15.90	ETHYL STEARATE	88.10
6	34.706	3535171	0.91	1202698	1.36	Ethyl 9.cis.,11.trans.-octadecadienoate	67.10
7	36.354	2057427	0.53	737601	0.84	2-Hydroxy-(Z)9-pentadecenyl propanoate	57.05
8	36.640	22758772	5.86	4734760	5.36	9-TERT-BUTYL-TRICYCLO[4.2.1.1 2,5]DECANE-9,10-DIOL	167.20
9	36.697	16391862	4.22	6547908	7.41	ETHYL 9-OXONONANOATE	57.05
10	37.034	21765206	5.60	7546048	8.54	ETHYL PENTADECANOATE	88.10
11	37.495	24360720	6.27	6137286	6.95	OXACYCLOHEXADECAN-2-ONE	55.05
12	37.575	3938579	1.01	1388363	1.57	1-Dimethyl(butyl)silyloxydecane	258.25
13	40.152	4596160	1.18	1821112	2.06	OCTADECANOIC ACID, ETHYL ESTER	88.10
14	43.069	1208389	0.31	517522	0.59	Ethyl tetracosanoate	88.10
		388471599	100.00	88325303	100.00		

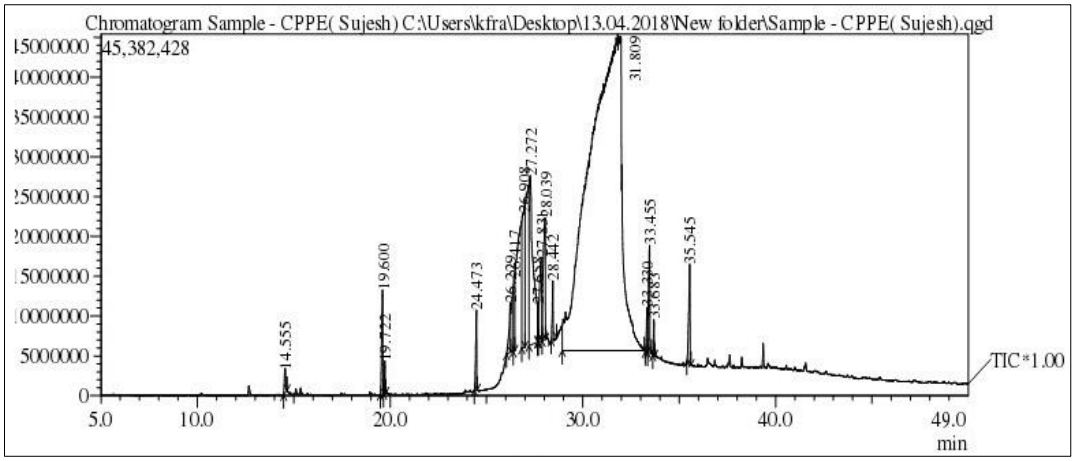

Fig 11: shows the chromatogram of CPPE extract

Table 5: shows GC-MS profile CPPE extract

Peak Report TIC							
Peak#	R.Time	Area	Area%	Height	Height%	Name	Base m/z
1	14.555	16260331	0.29	3055181	1.55	1-DODECANOL	55.10
2	19.600	49430160	0.87	13048119	6.60	2-Propenoic acid, tridecyl ester	55.05
3	19.722	10767616	0.19	4054151	2.05	Propanoic acid, decyl ester	57.05
4	24.473	32205264	0.57	10040131	5.08	HEXADECANOIC ACID, METHYL ESTER	74.05
5	26.229	36692907	0.65	6261799	3.17	Propanoic acid, 3-mercapto-, dodecyl ester	57.10
6	26.417	45832822	0.81	9249270	4.68	PALMITIC ACID	74.05
7	26.908	161917360	2.86	16889320	8.54	3-PENTANOL, 2,3-DIMETHYL-	73.05
8	27.272	307936563	5.44	21261084	10.76	HEXADECANOIC ACID	73.05
9	27.658	11255785	0.20	4780800	2.42	n-Decanoic acid	60.05
10	27.831	83795047	1.48	10532951	5.33	Methyl 10-trans,12-cis-octadecadienoate	67.05
11	28.039	114550385	2.02	15304446	7.74	9-OCTADECENOIC ACID (Z)-, METHYL ESTER	55.10
12	28.442	20527824	0.36	7345026	3.72	OCTADECANOIC ACID, METHYL ESTER	74.05
13	31.809	4625955100	81.72	39691941	20.08	cis-9-Hexadecenal	55.05
14	33.330	19463768	0.34	5617520	2.84	7,10-Hexadecadienoic acid, methyl ester	67.05
15	33.455	58394774	1.03	13484977	6.82	9-Octadecenamide, (Z)-	59.05
16	33.683	11836486	0.21	4509325	2.28	Octadecanamide	59.05
17	35.545	53839774	0.95	12530086	6.34	1,2-BENZENEDICARBOXYLIC ACID	149.05
		5660661966	100.00	197656127	100.00		

About 14 phytochemical compounds were identified in the CPEA extract and about 17 constituents were identified in the CPPE. Names of the phytoconstituents along with the molecular formula, molecular weight details are all listed in the tables (Table. 4 and 5). From the result it was observed that in CPEA extract ethyl-9-octa decenate, and hexadecanoic acid were found to be the major component followed by ethyl stearate and ethyl pentadeconate. In the CPPE the major component was cis-9-hexadecenal, and hexadecanoic acid. The minor components were 2, 3, dimethyl-3-pentanol and 9-octadecenamide. Recent studies has proved that Cyclea peltata possess anti-oxidant [11], anti-diuretic [12] hepatoprotective [13], hyperlipidemic [14] anti-diabetic activites [15] etc. It is reported that the tuberous root has the ability of treating nephrolithiasis [16] and type II diabetes [17]. These pharmacological action may be due to these various phytoconstituents.

4. Conclusion

Cyclea peltata is a well known drug cited in most of the ancient ayurvedic classics like Charaka Samhita, Sushruta Samhita and Astangahridya. It is also used by folk medicinal practisioners for treating various ailments. No much research has been done on it's phytochemical aspects. In the present study about 14 and 17 phytoconstituents hae bean identified in the ethyl acetate and pet. Ether extract of root of Cyclea peltata respectively.

These identified compound may have extensive therapeutic activities which may be the reson for it's pharmacological importance. Hence it is suggested that further isolation of compounds and screening for specific bioactivity can be done for developing novel therapeutic agents to treat various diseases.

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