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Research Article

Pharmacognostical and Phytochemical Studies on *Cayratiapedata (Lam)*

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ABSTRACT

Cayratia pedata (Lam.) of family Vitaceae is a woody climber seen widely distributed among most of the tropical Asian countries. Ethnopharmacological literature describes many of its medicinal uses.The current study aims on investigating some the pharmacognostical and phytochemical parameters of the leaves, which may complement some of the findings about the pharmacologic effects of the plant. The preliminary phytochemical analysis of various extracts of the leaves showed that it contains carbohydrates, tannins and phenolic compounds, terpenes, sterols, alkaloids and flavonoids. Pharmacological and biochemical investigations done on the leaf extracts of this plant by the author suggests that it possess antiinflammatory, anti-arthritic, anthelmintic properties. Moreover, it can down regulate expressions of inflammatory mediators like COX, iNOS and TNFα. (these findings were published by the researcher elsewhere). The terpenes rich fraction of the leaf was studied using column chromatography backed by TLC analysis revealed the presence some important components, which were isolated by preparative TLC. Detailed analysis of these components by HPTC, GCMS and NMR studies were conducted. It was found that compounds like citral, isopuligol, Limonene 1,2-epoxide, Linalyl anthranilate,Verbenol, delphidine, (+)-tans, trans caranol and diethyl Butane-di-oic acid are present in the leaf extracts. It is already established that these compounds contribute to antiinflammatory and anti-arthritic activities of many plants. It is also established that some terpenes can reduce expression of inflammatory mediators.

Key words: *Cayratia pedata*, pharmacognostical, .phytochemical

INTRODUCTION

Cayratia pedata (Lam)., family Vitaceae, is a large, but weak woody climber naturalized tropical evergreen and semi-evergreen forests of India, Andaman-Nicobar Islands, Sreelanka and Malaysia. It is common in shrubberies, hedges and waste places throughout the various parts of India, including Bihar, Orissa, West Bengal, Assam and Kerala.[1,2] In folklore medicine, *C.pedata* is used for a variety of ailments. Its mature leaves with shavings of Indian nut remove scabies. Leaves mixed with oil and boiled are used to cure wounds. The juice mixed with lime cures populas in skin or tongue.[3]When used as a poultice the leaves are rubefaceint. Roots are astringent. Ground with black pepper applied to boils.[4] Tuberous roots dried and powdered, mixed with sugar given as a medicine to cure swelling and in fevers. Overdose act as emetic. Young shoots powdered and fried in coconut oil given to children to remove intestinal worms, vomiting and diarrhea.[5] Efforts to explore and evaluate various pharmacologic activities of this plant have already been done by the researcher and the findings were published elsewhere. It was found that the ethanolic extracts of C.pedata possess significant antiinflammatory, antipyretic. anthelmintic and cytotoxic potential in cultured cells. It can suppress expression of LOX, COX-2, iNOs and TNF ∞.It was also observed that the extract has got significant effects on smooth muscle contraction .

The present study was aimed to evaluate some of the pharmacognostical parameters of C.pedata and to find out various active constituents present in it.

MATERIALS AND METHODS

Pharmacognostical studies[6,7]

Macroscopy: Macroscopical studis of various parts of *C.pedata were* carried out by observing and recording the colour, odour, taste etc

Microscopy: Microscopic examination of the various plant parts is important in pharmacognostical studies. In addition to providing information regarding the cellular organization, they also provide information regarding various phytoconstituents present in the plant and also give an idea where they are located. Microscopic features of *C.pedata* leaves were studied by cutting thin sections and then clearing them with chloral hydrate solution. The sections were then stained using various stains like Phloroglucinol-HCl, saffranin, iodine solution and ferric chloride solution.

Ash values: The estimation of ash values is important in drug standardization because it gives an idea about the content of fibre and inorganic matter present in the plant. This estimation is done in air dried samples of the plant or plant part by incinerating it to a constant weight and weighing.

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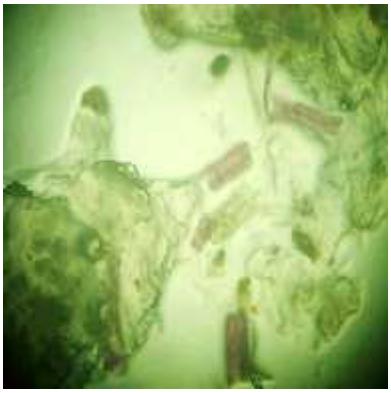


Development, Detection and Quantitation


Cayratia pedata -microscopy leaf:

Ash values

	Cayratiapedata
Total ash	9.62-10.5 %
Water soluble ash	2.4-3.7 %
Acid insoluble ash	3.2-3.9%

Loss on drying

Plant	*Cayratiapedata*
Loss on drying	

Loss on drying: It was estimated using 1g of the air-dried and powdered samples of *C.pedata* leaves taken in previously weighed crucible. It was then dried in a hot-air oven for one hour at 105 ^{o}C. After drying the crucible was closed and kept in desiccator to cool to room temperature. The crucible was then weighed. The process was repeated until constant weight obtained.

Phytochemical Studies

Collection, identification, drying and preparation of plant material for extraction: *Cayratia pedata* was collected from Palode village of Thiruvananthapuram district, during the month of June-July and identified and authenticated by taxonomists. (ARI, Poojappura,)The leaves were separated from the plant parts and were cleared off from external contaminants by careful hand picking, dried under shade at room temperature for two weeks and powdered. The coarse powder was used for extraction.

Extraction[8]: 100 g of the coarse powder were inserted into a soxhelt extractor and pre-extracted with petroleum ether (40-60) by hot continuous percolation process (5 cycles a day for three consecutive days). The marc was further extracted by 95 % alcohol till the extractives become almost colourless. The combined extractives were then evaporated in-vaccuo till a soft extract was obtained.

Fluorescence analysis[9]: Fluorescence characteristics of the powdered leaf were performed by treating it with different chemical reagents and were observed in daylight and UV light (254nm and 365nm).

Analysis of Phytoconstituents: The extract were screened for the presence of the following phytoconstituents carbohydrates, glycosides, alkaloids, sterols, fixed oils,

Fluorescence analysis

Reagent	*C.pedata*		
	Daylight	254 nm	365 nm
No reagent	Green	Bluish green	Grey-blue
50%KOH	green	Green	Light green
1M H2SO4	green	Dark green	Green
1M HCl	green	Light green	Light green
1M HNO3	green	Light green	Light green
50%H2SO4	green	Dark green	Light green
Glacial Acetic Acid	green	Light green	Light green
Aq.NaOH	green	Bluish green	Grey green
MethanolicNaOH	green	Bluish green	Grey green
Acetone	Green	Yellowish green	Grey-green
FeCl3	Light yellow	Brow-yellow	Yellowish green
Iodine	Dark green	Dark brown	Dark green
Ethanol	green	green	green

TLC profile[11]

Fraction: Ethyl acetate 100%
Solvent system: n-Hexane: Chloroform:Ethanol= 2:1:1
Plate:TLC silica gel60F254(Merck,Germany)

No of spots	RF value
Spot-1	0.94
Spot-2	0.78
Spot-3	0.63
Spot-4	0.5

Fraction: Ethyl acetate + ethanol(50:50)
Solvent system: n-Hexane: Acetone:Ethylacetate= 4:1:1
Plate:TLC silica gel60F254(Merck,Germany)

	RF value
Spot-1	0.95
Spot-2	0.91
Spot-3	0.76
Spot-4	0.69
Spot-5	0.64
Spot-6	0.55

saponins, flavonoids, fats, phenolic compounds, tannins, terpenes, proteins and amino acids.

Column chromatography: Details of column chromatography of alcoholic extract: column chromatography was done using a 60cm column with silicagel(60-120) mesh size and 7 g extract. The column was eluted with solvents of increasing polarity and fractions were pooled with help of parallel TLC analysis..

HPTLC profile: 5 µl aliquots of each of the extracts were separately applied on aluminium plates precoated with Silica gel 60 F254 HPTLC plates, 10 × 10 cm (Merck, Darmstadt, Germany) with the help of Camag Linomat-V applicator. Fraction D isolated from column chromatography was applied on 6 mm wide band using Camag Linomat-V automated TLC applicator with the

Fraction: Ethyl acetate+ Ethanol(50:50)
Mobile phase: n-Hexane: Ethanol:Ethylacetate= 8:1:1
Plate:TLC silica gel60F254(Merck,Germany)

	RF value
Spot-1	0.85
Spot-2	0.75
Spot-3	0.64
Spot-4	0.59
Spot-5	0.55
Spot-6	0.5
Spot-7	0.45

Fraction: Petroleum ether
Solvent system: n-Hexane: Toluene:Ethylacetate= 4:5:1
Plate:TLC silica gel60F254(Merck,Germany)

	Solute front	Solvent front	RF value
Spot-1	8.1	8.4	0.96
Spot-2	6.3	8.4	0.75
Spot-3	5.7	8.4	0.68
Spot-4	4	8.4	0.48
Spot-5	3.2	8.4	0.38
Spot-6	2.8	8.4	0.33
Spot-7	2.5	8.4	0.29
Spot-8	1.8	8.4	0.21

nitrogen flow providing a delivery speed of 150 nL/sec from syringe.

Development, Detection and Quantitation: After sample application, plates were developed in a Camag twin through glass tank pre-saturated with the mobile phase Toluene: methanol (7:3) for 20 min. The plate was developed horizontally in Camag horizontal developing chamber (10 × 10 cm) at the room temperature. After developing the plate was dried and was observed under UV-366 nm light in Camag UV cabinet. The corresponding digital scanning profiling was carried out with a Camag TLC scanner III fitted with winCATS-V1.2.3 software at a single wavelength 260 nm.

GCMS Analysis: GC-MS analysis of the ethanolic extract was carried out by split less injection of 1µL of the extract on a Hewlett Packard 6890 gas chromatograph fitted with an HP-MS cross linked 5% PH ME siloxane, 30m x 0.32mm x 0.25µ capillary column, coupled with a model 5973 mass detector. GC MS operation condition: injector temperature 220˚C, transfer line 240˚C, oven temperature programme 60˚C - 243˚C (3˚C min^{-1}); carrier gas He at 1.4ml/min^{-1}

Mass spectra: Electron impact mode 70eV, ion source temperature 240˚C. The individual components were identified by NIST library

RESULTS AND DISCUSSION

Pharmacognostical studies

Macroscopy: The plants collected occurred as woody climbers with tuberous roots. The stems consist of branches, leaves and fruits. The taxonomic characteristics of the plants confirmed that the plant belongs to the family Vitaceae. The plant was found to be belonging to the genus *Cayratia* and species *pedata*. The identity of the specimen was confirmed by taxonomists in ARI, poojappura, Thiruvananthapuram.

Nature: woody climber.

Pubescence:Young stem, petioles, tendrils and peduncles are densely pubescent. lamina is sparsely hairy above, densely pubescent below.

Leaflets: 7-9, middle ones large, smaller towards sides

Shape: oblong-lanceolate shape.

Size: 5-15 X 2-4 cm.

Apex: acute

Base: acuminate. Side ones obliquely attenuate

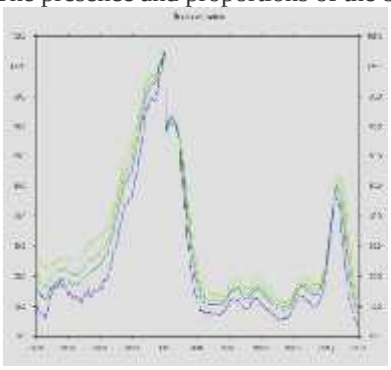
Margins: Densely serrate

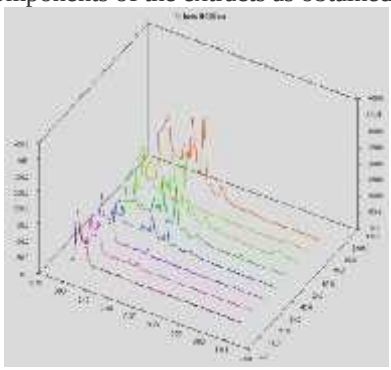
Flowers: monopetalous with five partite, arise in racemes from small dark green bud in axillary dense corymbs. Flowers have no smell.

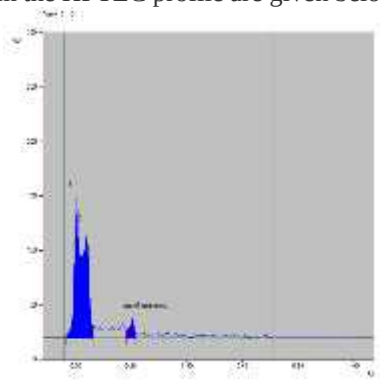
Peduncles: 2-3.5 cm long.

HPTLC profile

The presence and proportions of the 8 components of the extracts as obtained from the HPTLC profile are given below:



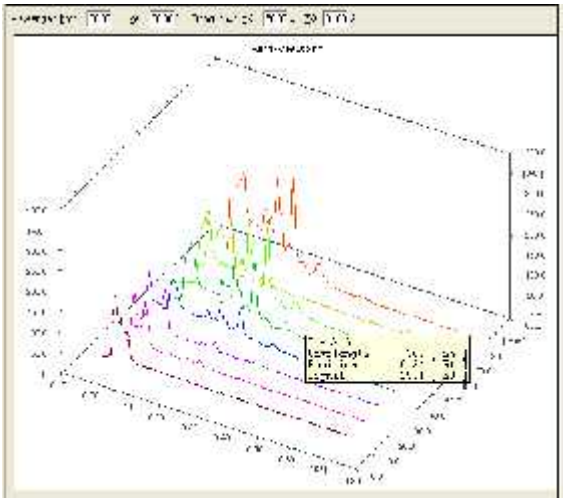




Scan settings

Slit dimension	5.00 x 0.45 mm, Micro
Optimize optical system for maximum	Light
Scanning speed	20 mm/s
Data resolution	100 pm/step

Measurement	
Wavelength	366 nm
Lamp	D2 W
Measurement type	Remission
Measurement mode	Absorption
Optical filter	Neutral filter
Detector mode	Automatic
Y-position for 0 adjust	5.0 mm
Track # for 0 adjust	1
Track start for quick scan	Automatic
Track end for quick scan	Automatic
Track # for quick scan	Automatic
Analog offset	0.0 %
Sensitivity	Automatic



Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.04 Rf	1.1 AU	-0.00 Rf	151.0 AU	55.77 %	0.02 Rf	72.3 AU	2707.8 AU	62.52 %	unknown *
2	0.02 Rf	72.7 AU	0.03 Rf	80.4 AU	33.29 %	0.07 Rf	8.3 AU	1187.4 AU	30.48 %	unknown *

Track 2, ID:

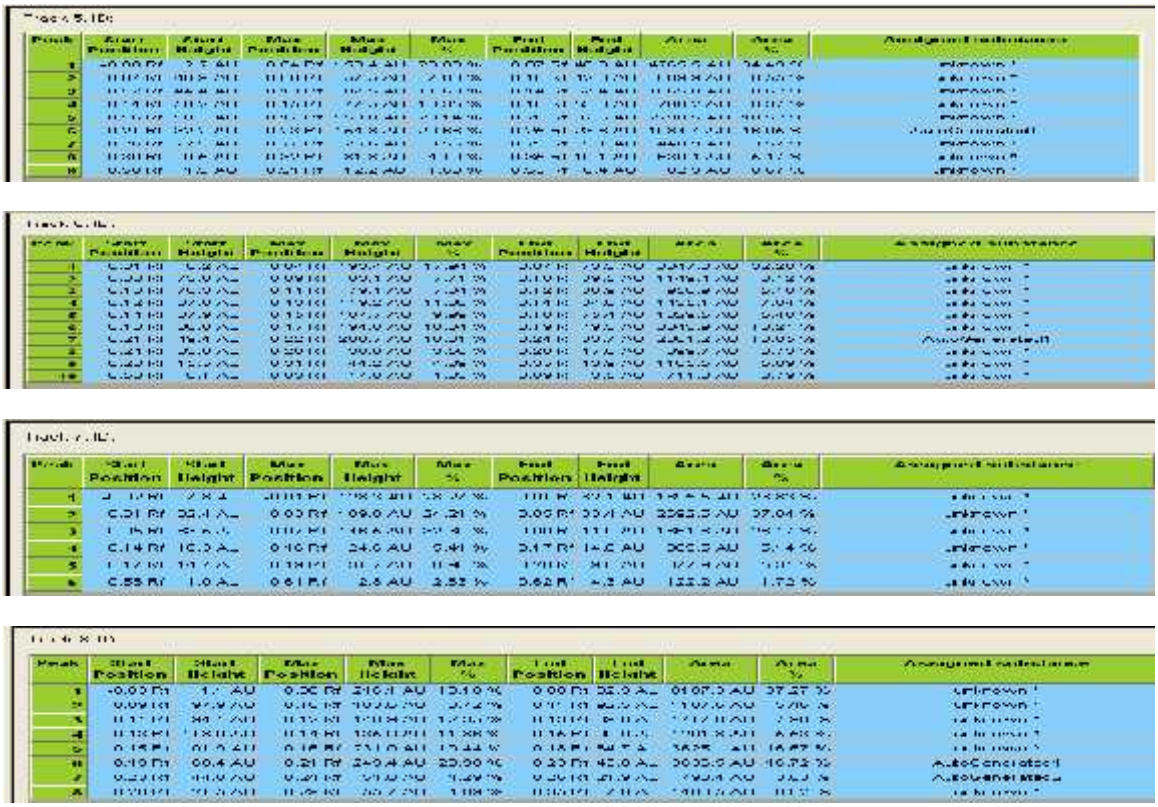
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.03 Rf	1.0 AU	0.01 Rf	130.0 AU	52.86 %	0.02 Rf	73.6 AU	1020.0 AU	40.24 %	unknown *
2	0.03 Rf	73.5 AU	0.04 Rf	97.4 AU	39.60 %	0.07 Rf	9.8 AU	1738.0 AU	44.20 %	unknown *
3	0.18 Rf	6.0 AU	0.20 Rf	18.5 AU	7.54 %	0.22 Rf	4.1 AU	240.4 AU	6.07 %	AutoGenerated3

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	-0.01 Rf	2.9 AU	0.00 Rf	144.1 AU	54.17 %	0.00 Rf	70.1 AU	3007.5 AU	52.99 %	unknown *
2	0.01 Rf	70.2 AU	0.02 Rf	112.1 AU	42.12 %	0.06 Rf	10.1 AU	1610.4 AU	28.38 %	unknown *
3	0.19 Rf	0.0 AU	0.20 Rf	24.1 AU	9.01 %	0.22 Rf	0.0 AU	1272.1 AU	22.42 %	AutoGenerated3

Track 4, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %	Assigned substance
1	0.01 Rf	0.5 AU	0.03 Rf	91.8 AU	35.21 %	0.07 Rf	20.5 AU	2650.8 AU	41.33 %	unknown *
2	0.12 Rf	36.8 AU	0.14 Rf	45.0 AU	17.23 %	0.14 Rf	32.3 AU	646.3 AU	10.08 %	unknown *
3	0.15 Rf	32.4 AU	0.17 Rf	43.1 AU	16.51 %	0.18 Rf	18.0 AU	478.2 AU	8.32 %	unknown *
4	0.18 Rf	20.7 AU	0.20 Rf	60.3 AU	23.09 %	0.22 Rf	8.0 AU	1223.6 AU	19.07 %	unknown *
5	0.24 Rf	18.4 AU	0.25 Rf	85.8 AU	32.88 %	0.26 Rf	9.8 AU	1112.2 AU	17.34 %	AutoGenerated1
6	0.31 Rf	7.1 AU	0.33 Rf	17.4 AU	6.70 %	0.35 Rf	7.0 AU	267.5 AU	4.21 %	unknown *



Pedicels: 0.3 to 0.4cm.
Stamens: 4, attached to the disc. Ovary 2 celled. Ovules: 2 in each cell; style: subulate. Fruits: Grapes like, round-plain, berries about 05-1 cm diameter and bilobed with 2-4 seeds

Microscopy: Dried powder of the leaves and arial pars shows lignified fibres, glass like thick crystals of calcium oxalate, multi-cellular elongated and covering trichomes, xylem elements and fragments containing collenchyma cells.

Cayratia pedata -microscopy leaf: A C.S of the leaflet through midrib shows cuticle followed by a single layer of flattened epidermal cells with uni seriate multi cellular and covering trichomes. Single layer of tubular palisade cells containing chlorophyll are seen, followed by aerenchyma in the lamina.

In the midrib region xylem elements with lignified walls and phloem elements are seen. Below the vascular bundles, collenchyma with unevenly thickened cell walls and lower epidermis are seen. Section mounted in ferric chloride solution appeared as greenish yellow and sections stained with Iodine showed bluish spots.

Phytochemical Studies

Extraction: The yield of extraction of powdered leaves with petroleum ether was 4.7 % and that with ethanol (95%) was 3.46%.W/W

Analysis of Phytoconstituents: Preliminary phytochemical analysis of various extracts of leaves of C.pedata showed the presence of carbohydrates, flavonoids, tannins and phenolic compounds and terpenes.

Column chromatography[10]: 620 fractions were collected by column chromatographic technique. From these 8 pooled fractions were separated for further detailed analysis.

GCMS Analysis: The compounds identified by GCMS analysis were: Verbenol, Butanedioic acid, Citral, Isopulegol, Linalylanthranilate, , Bornol, (+) trans, trans caranol, and thajupsol.

CONCLUSION

Observations of this study revealed that the leaf of C.pedata contains constituents like flavonoids, alkaloids, tannins, carbohydrates and terpenes. The terpenes isolated was found to be Verbenol, Butanedioic acid, Citral, Isopulegol, Linalyl anthranilate, Bornol, (+) trans, trans caranol, and thajupsol. Antiinflammatory activity of many terpenes and flavonoid containing plants have already been evaluated and published by several authors.[14,15,16]. The presence of these phytocomponents, thus partly substantiates the antiinflammatory activity of C.pedata.

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