Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1637-1642
Received: 09-01-2019
Accepted: 10-02-2019

Aswathy TR
Department of Computational Biology and Bioinformatics, University of Kerala, Kerala, India

Gayathri E
Department of Bioinformatics, School of Life Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Praveen J
Department of Bioinformatics, School of Life Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

Achuthsankar S Nair
Department of Computational Biology and Bioinformatics, University of Kerala, Kerala, India

Sugunan VS
Department of Computational Biology and Bioinformatics, University of Kerala, Kerala, India

Correspondence
Aswathy TR
Department of Computational Biology and Bioinformatics, University of Kerala, Kerala, India

Phytoprofiling of medicinal plant *Cayratia pedata* by qualitative and quantitative method

Aswathy TR, Gayathri E, Praveen J, Achuthsankar S Nair and Sugunan VS

Abstract
Cayratia pedata is an indigenous medicinal plant commonly known as Birdfoot Grapevine. The leaves of this plant have been used as a traditional medicine for the treatment of ulcers, diarrhea and inflammation. The crude extracts of the plant has anti-microbial, anti-ulcer, anti-inflammatory and anti-diarrheal properties. The present study reports on the bioactive principles obtained from *Cayratia pedata* leaves through extraction, isolation and identification. The phytochemicals screened and quantitative analyses were done by biochemical tests. The chemical composition of the plant leaves extract was resolved by Gas Chromatography – Mass Spectral (GC-MS) analyses. The preliminary analyses reveal the presence of tannins, flavonoids, terpenoids, alkaloids, carbohydrate, saponins, steroids, quinines, phenols, proteins, oils and fats, phytosterols, coumarins and phlobatannins. The GC-MS results, confirmed that the bioactivity of *Cayratia pedata* depends on several active principles of which have detected thirty five different phytochemicals that belongs to acid, ester, alcohol and ether groups.

Keywords: *Cayratia pedata*, phytochemicals, bioactive principles, GC-MS

Introduction
Plants have been used as remedies for thousands of years and about 80% of the world's population use plants and their products to treat several diseases. Two constituents of phytochemicals are recognised: primary and secondary. Primary constituents include chlorophyll, amino acids, proteins and common sugars while the secondary constituents are flavonoids, essential oils, tannins, terpenoids, alkaloids and phenolic compounds (Edeoga *et al*., 2005; Krishnaiah *et al*., 2007) [10, 19]. These metabolites have been demonstrated to have several biological and medicinal values such as anti-inflammatory, anti-fungal, anti-bacterial, anti-constipative and anti- oxidant activities (Ferdous *et al*., 1992) [12]. About one half of the drugs that reached the market during the last 20 years are derived directly or indirectly from these small molecules.

Cayratia pedata commonly known as Birdfoot Grapevine is an indigenous South Indian medicinal herb belonging to the family Vitaceae. It is included in the IUCN list of vulnerable plants. The leaf of this plant is traditionally used as a medicine for the treatment of ulcers, diarrhoea and inflammation. The paste of *Cayratia pedata* has been applied by tribals as an early cure for wounds and migraine. Previous studies report that the crude extracts of the plant has anti-microbial (Nayak *et al*., 2014) [21] anti-ulcer (Karthik *et al*., 2010) [16], anti-inflammatory (Rajendran *et al*., 2011) [27], anti-nociceptive (Rajendran *et al*., 2011) [28], anti-arthritic (Selvarani *et al*., 2014) [23], anti-oxidant (Selvarani *et al*., 2014) [23] and anti-diarrhoeal (Karthik *et al*., 2014) [17] properties. We investigated and evaluated the presence of active principles from *Cayratia pedata* leaves extract by qualitative and quantitative methods.

Materials and Methods
Plant sample
The leaves of *Cayratia pedata var.glabra* (Voucher No: KUBH10096) was collected from the local area of Trivandrum, Kerala during January 2015 and identified by the Taxonomist, Department of Botany, University of Kerala.

Extraction of biochemicals from plant leaves using Soxhlet apparatus
The plant leaves were dried under shade and ground to a fine powder with a mechanical grinder. 20 gm of the finely ground leaves were placed in a porous bag made of strong filter paper, and placed in the extraction chamber of the Soxhlet apparatus and extracted with 100% of methanol by refluxing for 2 days. The solvent was evaporated and the extract stored at 4^0C (Hossain & Nagooru, 2011) [14].

Qualitative analyses of phytochemicals by Biochemical tests

Biochemical tests to check for the presence of phytoconstituents such as tannins, flavonoids, terpenoids, alkaloids, carbohydrates, saponins, quinones, phenols, oils and fats, phytosterols, coumarins, phlobatannins and anthraquinone were done according to standard procedures (Evans, 2009; Mangathayaru, 2013) [11, 20].

Quantitative analysis of photochemicals

Estimation of alkaloids

The alkaloids were estimated by spectrophotometry using Dragend offs reagent (Sreevidya & Mehrotra, 2003) [25]. 10 mg of the sample was accurately weighed and made up to 1ml with DMSO. The sample was centrifuged for 10 minutes at 3000rpm to remove suspended particles. 0.5 ml extract was mixed with 1ml of 0.1 N. HCl. Then 0.25 ml of Dragendorff's reagent was added to the mixture for precipitation and the precipitate was centrifuged for 5 minutes at 3000 rpm. This precipitate was further washed with 0.25 ml of ethanol. The filtrate was discarded and the residue was treated with 0.25 ml of disodium sulphide solution (1%wlv). The brownish black precipitate formed was centrifuged (5 min 3000 rpm) and the residue was dissolved in 0.2 ml of con. Nitric acid, mixed with 1ml 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)

Estimation of total flavonoids

10 mg of the sample was accurately weighed and made up to 1ml with DMSO. 0.5ml of extract stock solution, 1.5 ml methanol, 0.1 ml aluminium chloride, 0.1 ml potassium acetate solution and 2.8 ml distilled water was added and mixed well. Sample blank was prepared in similar way by replacing aluminium chloride with distilled water. Sample and sample blank of all four extracts were prepared and their absorbance was measured at 415 nm. Quercetin was used as standard to make the calibration curve. 10 mg of quercetin was dissolved in 1 ml of methanol and then diluted to 100, 200, 400, 800, and 1000 µg/ml (Hossain & Rahman, 2011) [13].

Estimation of total phenols

Total phenolic content of extract was estimated by Folin-Ciocalteau method (Smith *et al.*, 1985) [24]. 100µl sample was pipetted out into a test tube and 5ml of Folin-Ciocalteau reagent was added. After 5 minutes, 4ml of sodium carbonate solution was added and incubated at room temperature for 2 hours. Then, absorbance was measured at 750 nm using a UV-VISIBLE spectrophotometer and the values obtained were interpreted from the standard graph. Gallic acid was used as standard (10 mg/ml). The results were determined from the standard curve and were expressed as gallic acid equivalent (mg/g of extracted compound).

Identification of Phytochemicals by Gas Chromatography and Mass Spectroscopy (GCMS)

The GC-MS analysis was carried out on a GC Clarus 500 Varian, USA system comprising a AOC-20I auto sampler and gas chromatograph interfaced to a mass spectrum analyser employing the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm I.D ×1 µ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV. Helium (99.999%) was used as carrier gas at a constant flow of 1ml/min. Injection volume was 0.5 µ l (split ratio of 10:1) with an injector temperature of 250°C and ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; with a scan interval of 0.5 seconds and fragments from 45 to 450 Da were recorded. Total GC running time was 46 min.

Result and Discussion

Qualitative analyses of phytochemicals by Biochemical tests

Biochemical tests carried out on the extracts of the dried leaves of *Cayratia pedata* showed the presence of tannins, flavonoids, terpenoids, alkaloids, carbohydrate, saponins, steroids, quinines, phenols, proteins, oils and fats, phytosterols, coumarins and phlobatannins. These phytochemicals have scientifically confirmed health benefits for the prevention and treatment of different diseases. The qualitative analysis of ethanolic extract of *Cayratia pedata* reported by Stanley *et al*., (2012) [26] shows the presence of the tannins, carbohydrate, flavonoid, alkaloids, phenolic components, terpenoids, and steroids.

Quantitative analysis of phytochemicals

Estimation of total alkaloids

The amount of alkaloids present in the methanolic extract of *Cayratia pedata* leaves was determined by spectrophotometric method. The alkaloids content of the extract was 4.460 mg in 10 mg of the extract expressed as standard equivalent per gram. The result shows that the plant leaves extract was rich in alkaloids. Alkaloids are pharmacologically and therapeutically important group of compounds present in natural sources. Various works report that alkaloids have anti-bacterial (Cushnie *et al*., 2014) [7], anti-cancer (Kittakoop *et al.*, 2014) [18], anti-analgesic (Wang *et al.*, 2010) [29] and vasodilator (Chiou *et al*., 1996) [4] properties also.

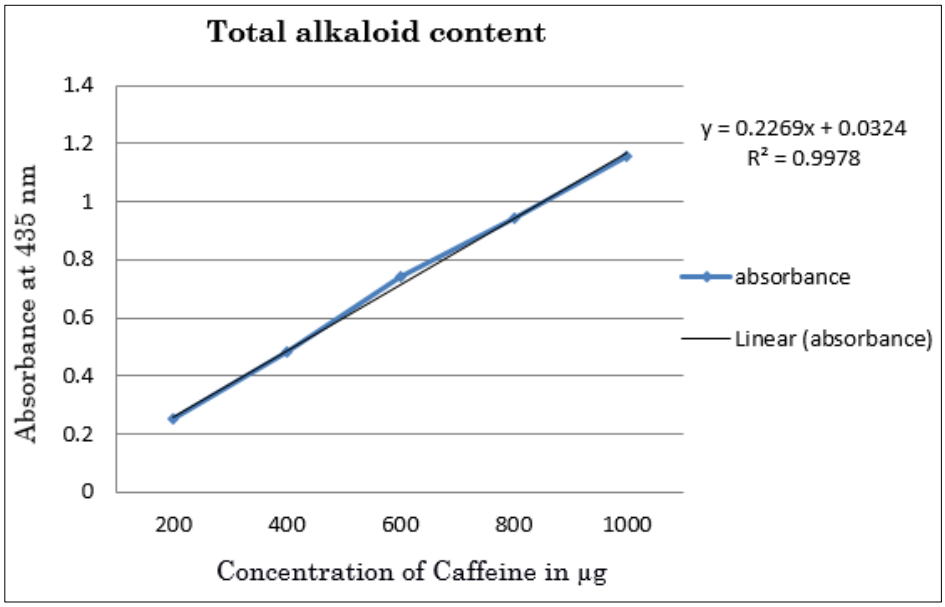

Fig 1: Calibration graph for total alkaloid content

Estimation of total flavonoid

The total flavonoid content for methanol extracts was measured with the aluminium chloride colorimetric assay using quercetin as the standard. The total flavonoids contents of the methanolic extract of *Cayratia pedata* leaves extract in terms of quercetin equivalent were 2.51 mg in 10 mg of extract powder respectively. Flavonoids are the most important group of compound present in all plant parts, especially the photosynthesising plant cells. Flavonoids have therapeutic importance like anti-ulcer (Smith 1988) [5], anti-oxidant (Bors *et al*., 1990) [2], vasodilatory (Duarte *et al*., 1993) [9], anti-inflammatory (Hoult *et al*., 2004) [15] and anti-bacterial (Cushnie *et al*., 2011) [8] activity.

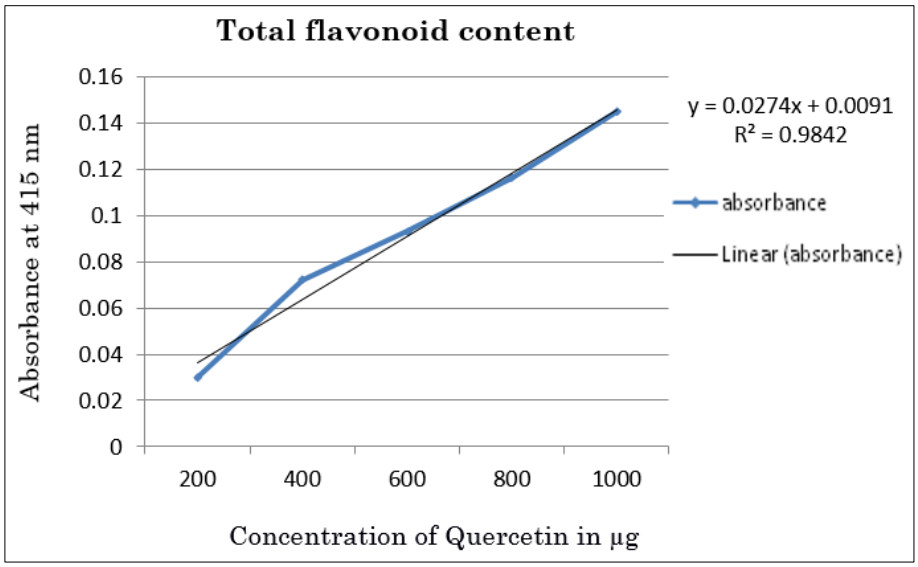

Fig 2: Calibration graph for total flavonoid content

Estimation of total phenol content

The amount of total phenolic content was estimated with the Folin-Ciocalteu reagent. Gallic acid was used as standard. The total phenolic contents of the methanolic extract of *Cayratia pedata* leaves extract in terms of gallic acid equivalent was 1.91 mg in 10 mg of extract powder. Phenolic compounds are the major secondary metabolites found in the plants. It plays an important role in defence response in plants. Previous studies reported that the phenolic compounds have been used as medicines against diabetes (You *et al*., 2012) [30], inflammation (Alarcon *et al*., 2005) [1], cancer (Cai *et al*., 2004) [3], heart disease (Covas, 2008) [6] and oxidative stress (Rice-Evans *et al*., 1997) [22].

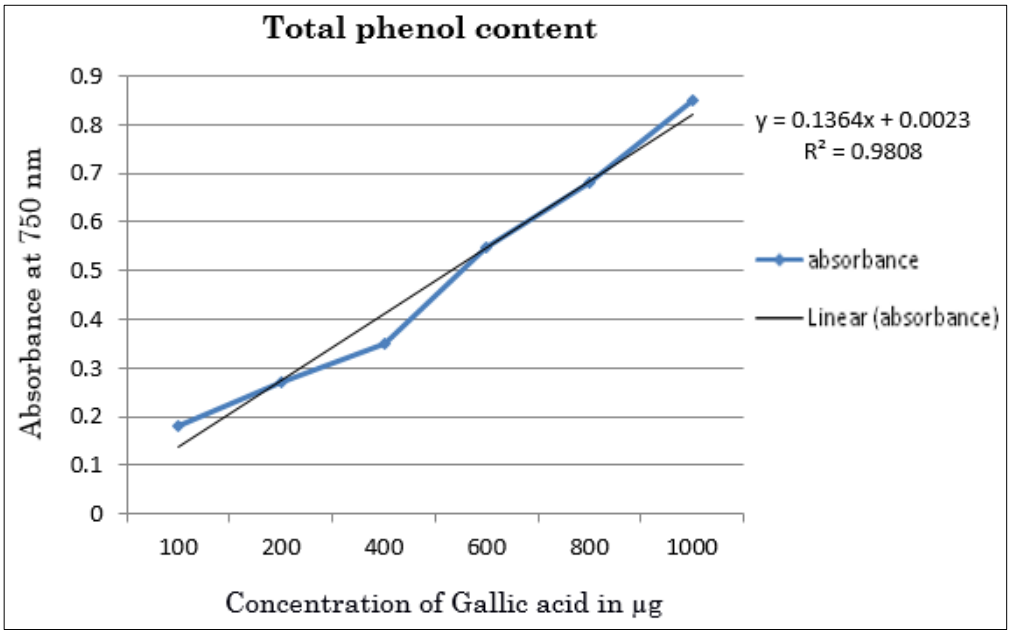

Fig 3: Calibration graph for total phenol content

Identification of Phytochemicals by Gas Chromatography and Mass Spectroscopy (GCMS)

Analysis of the methanol extract of the plant leaves using GCMS showed the presence of thirty five compounds.

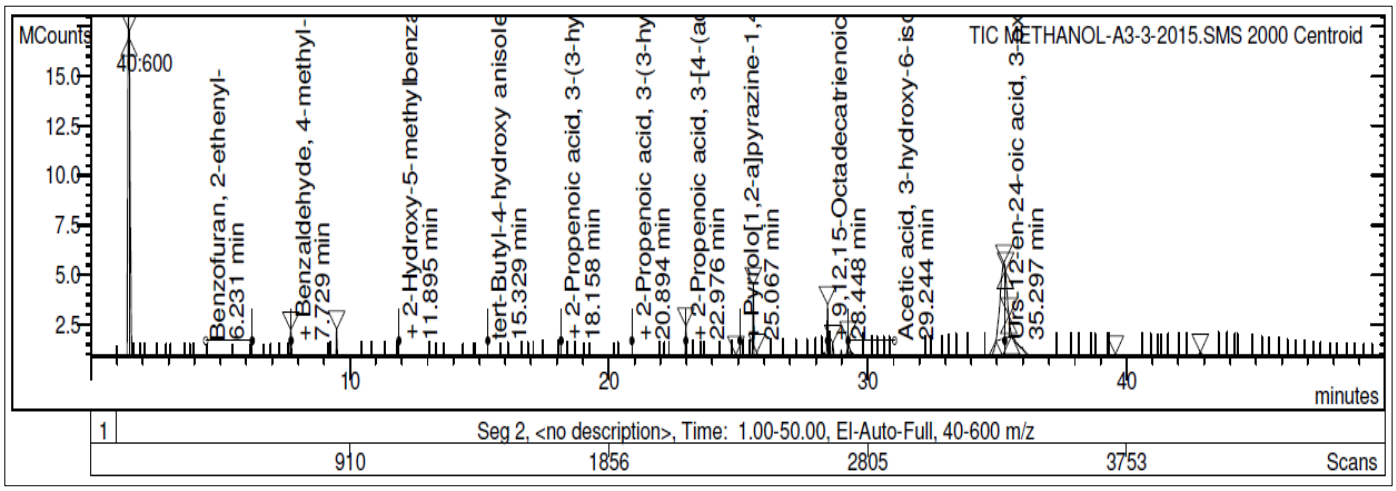

Fig.4: Analysis of Compounds present in the *Cayratia pedata* by GC-MS analysis

The spectrum (Fig.4) shows thirty five prominent peaks in the retention time range 6.231 - 42.849. Urs-12-en-24-oic acid, 3-oxo-, methyl ester showed largest peak area with a retention time of 35.297 (MW 468.7 g/mol). The Second prominent peak showed 4-Methyl benzaldehyde (MW 120.1 g/mol). Other therapeutically important phytochemicals like Megastigmatrienone, 3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol, 9, 12, 15-Octadecatrienoic acid, methyl ester, Phytol and 4-Hydroxy-2-methylacetophenone were also present. The detailed descriptions of the phytochemicals are given in the table1. The compounds identified by GC-MS analysis have many biological important properties. The activities listed are based on literature review and Dr. Duke's Phytochemical and Ethnobotanical Databases.

Table 1: Details of phytoconstituents from *Cayratia pedata* leaves extract.

S. No	RT	Compounds	Molecular Formula	Molecular Weight	Reported activities
1	6.23	Benzofuran, 2-ethenyl-	$C_{10}H_8O$	144.16	No activity reported
2	7.72	Benzaldehyde, 4-methyl-	C_8H_8O	120.14	No activity reported
3	8.15	2-Benzyl-2-methyl-1,3-oxathiolane	$C_{11}H_{14}OS$	194.29	No activity reported
4	9.28	1-Ethyl-1-isopropoxy 1-silacyclopentane	$C_9H_{20}OSi$	172.34	No activity reported
5	9.48	4-Hydroxy methylacetophenone	$C_9H_{10}O_2$	150.17	No activity reported
6	10.1	alpha.-D-Xylofuranoside, methyl	$C_6H_{12}O_5$	164.15	No activity reported
7	11.6	L-Proline, 1-acetyl-, methyl ester	$C_8H_{13}NO_3$	171.2	No activity reported
8	11.8	2-Hydroxy-5-methylbenzaldehyde	$C_8H_8O_2$	136.14	No activity reported
9	13.1	2,6-Difluorobenzoic acid	$C_7H_4F_2O2$	158.10	No activity reported
10	15.3	tert-Butyl-4-hydroxy anisole	$C_{22}H_{32}O_4$	360.48	Anti -oxidant

11	16.3	Ethyl N-(o-anisyl) formimidate	$C_{10}H_{13}NO_2$	179.21	No activity reported
12	17.3	Megastigmatrienone	$C_{13}H_{18}O$	190.28	No activity reported
13	17.5	Tricyclo[4.3.1.1(3,8)]undecane, 1-methoxy-	$C_{12}H_{20}O$	180.28	No activity reported
14	18.1	2-Propenoic acid, 3-(3-hydroxyphenyl)-, methyl ester	$C_{10}H_{10}O_3$	178.18	Anti-oxidant
15	18.2	4,4,5,8-Tetramethylchroman-2-ol	$C_{13}H_{18}O_2$	206.28	No activity reported
16	18.7	2-Heptanone, 6-(3,5-dimethyl-2-furanyl)-	$C_{14}H_{22}O_2$	222.32	No activity reported
17	19.6	2-Propenoic acid, 3-[4-(acetyloxy)-3-methoxyphenyl	$C_{12}H_{12}O_5$	236.22	No activity reported
18	21.3	Ethanone, 1-[1-hydroxy-3,3-dimethyl-	No details reported		
19	22.6	2-(3-Isopropyl-4-methyl-pent-3-en-1-ynyl)-2-methyl-cyclobutanone	$C_{14}H_{20}O$	204.30	No activity reported
20	23.4	Cyclopropanenonanoicacid, 2-[(2-butylcyclopropyl)methyl]-, methyl ester	$C_{21}H_{38}O_2$		No activity reported
21	23.8	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296.53	Anti-microbial, Anti- inflammatory, Anti-cancer, Diuretic, Anti-oxidant
22	24.9	Pentadecanoic acid, 14-methyl-methyl ester	$C_{17}H_{34}O_2$	270.45	Anti-fungal, anti-bacterial
23	25.0	Pyrrolo 1 2-a pyrazine-1 4-dione hexahydro-3-(2-methylpropyl)-	$C_{11}H_{18}N_2O_2$	210.27	Anti-bacterial, Anti-cancer
24	25.7	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652.94	Hyaluronidase Inhibitor, Anti-oxidant, Cardio protective, Cancer preventive, Anti-infertility
25	26.3	3-Phenylbicyclo(3.2.2) nona-3,6-dien-2-on	$C_{15}H_{14}O$	210.271	No activity reported
26	27.0	Dasycarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326.43	Anti-bacterial activity
27	28.4	9,12,15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.45	Anti-inflammatory, Anti cancer, Hypocholesterolemic, preventive, Hepatoprotective, Anti-acne Nematicide, Insectifuge, Anti-histaminic, Anti-eczemic, Anti-androgenic, Anti-arthritic, Anti-coronary heart disease.
28	28.6	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl	$C_{12}H_{20}O$	180.28	Antimicrobial
29	28.9	Heptadecanoic acid, 15-methyl-, methyl ester	$C_{19}H_{38}O_2$	298.50	Antibacterial
30	29.2	Acetic acid, 3-hydroxy-6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-2-yl ester	$C_{17}H_{26}O_3$	278.38	No activity reported
31	35.0	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1-en-1-carboxaldehyde	$C_{23}H_{32}O$	324.5	Antimicrobial, Anti-inflammatory
32	35.2	Urs-12-en-24-oic acid, 3-oxo-, methyl ester	$C_{31}H_{48}O_3$	468.7	Antimicrobial
33	36.1	10-vinyl-19-Norpregn-4-ene-3,20-dione,	$C_{22}H_{30}O_2$	326.47	No activity reported
34	39.6	Docosahexaenoic acid, 1,2,3-propanetriyl ester	$C_{69}H_{98}O_6$	1029.51	No activity reported
35	42.8	4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-(1-methylethyl)-	$C_{20}H_{34}O_2$	306.48	No activity reported

Conclusion

The phytochemical screening of methanolic extract *Cayratia pedata* revealed the presence of various biologically important phytochemicals like tannins, flavonoids, terpenoids, alkaloids, carbohydrate, saponins, steroids, quinines, phenols, proteins, oils and fats, phytosterols, coumarins and phlobatannins. The quantitative analysis confirmed that the plant leaf extract was rich in alkaloids, phenols and flavonoids, several of which are pharmacologically important phytochemicals. The results showed the presence of thirty five compounds in the methanolic extract of the plant. Among the thirty five compounds identified, some of them are already known to possess medicinal properties. Hence it can be interpreted that *Cayratia pedata* could be a potential source for the development of drugs against several diseases.

References

1. Alarcon De La Lastra C, Villegas I. Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. Molecular nutrition & food research. 2005; 49(5):405-430.
2. Bors W, Heller W, Michel C, Saran M. Flavonoids as antioxidants: Determination of radical-scavenging efficiencies, In Methods in enzymology, Academic Press. 1990; 186:343-355.
3. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life sciences. 2004; 74(17):2157-2184.
4. Chiou WF, Liao JF, Chen CF. Comparative study on the vasodilatory effects of three quinazoline alkaloids isolated from Evodia rutaecarpa. Journal of natural products. 1996; 59(4):374-378.
5. Smith PC, Thomas P, Scurr JH, Dormandy JA. Causes of venous ulceration: a new hypothesis. British medical journal (Clinical research ed.). 1988; 296(6638):1726.
6. Covas MI. Bioactive effects of olive oil phenolic compounds in humans: reduction of heart disease factors and oxidative damage. Inflame Mo pharmacology. 2008; 16(5):216-218.
7. Cushnie TT, Cushnie B, Lamb AJ. Alkaloids: an overview of their antibacterial, antibiotic-enhancing and

antivirulence activities. International Journal of Antimicrobial Agents. 2014; 44(5):377-386.
8. Cushnie TT, Lamb AJ. Recent advances in understanding the antibacterial properties of flavonoids. International journal of antimicrobial agents. 2011; 38(2):99-107.
9. Duarte J, Perez-Vizcaino F, Zarzuelo A, Jimenez J, Tamargo J. Vasodilator effects of quercetin in isolated rat vascular smooth muscle. European journal of pharmacology. 1993; 239(1-3):1-7.
10. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African journal of biotechnology. 2005; 4(7):685-688.
11. Evans WC. Trease and Evans' Pharmacognosy E-Book. Elsevier Health Sciences, 2009.
12. Ferdous AJ, Islam SN, Ahsan M, Hasan CM, Ahmed ZU. *In vitro* antibacterial activity of the volatile oil of Nigella sativa seeds against multiple drug-resistant isolates of Shigella spp. and isolates of Vibrio cholerae and Escherichia coli. Phytotherapy Research. 1992; 6(3):137-140.
13. Hossain MA, Rahman SM. Total phenolics, flavonoids and antioxidant activity of tropical fruit pineapple. Food Research International. 2011; 44(3):672-676.
14. Hossain MA, Nagooru MR. Biochemical Profiling and Total Flavonoids Contents of Leaves Crude Extract of Endemic Medicinal Plant *Cordyline terminalis* L. Kunth. Pharmacognosy Journal. 2011; 3(24).
15. Hoult JRS, Moroney MA, Paya M. Actions of flavonoids and coumarins on lipoxygenase and cyclooxygenase, In Methods in enzymology. Academic Press. 1994; 234:443-454
16. Karthik P, Kumar RN, Amudha P. Anti diarrheal activity of the chloroform extract of Cayratia pedata Lam in albino wistar rats. Pharmacologyonline. 2011; 2:69-75.
17. Karthik P, Amudha P, Srikanth J. Study on phytochemical profile and antiulcerogenic effect of Cayratia pedata Lam in albino wistar rats. J Pharmacology online. 2010; 2:1017-1029.
18. Kittakoop P, Mahidol C, Ruchirawat S. Alkaloids as important scaffolds in therapeutic drugs for the treatments of cancer, tuberculosis, and smoking cessation. Current topics in medicinal chemistry. 2014; 14(2):239-252.
19. Krishnaiah D, Sarbatly R, Bono A. Phytochemical antioxidants for health and medicine a move towards nature. Biotechnology and Molecular Biology Reviews. 2007; 2(4):97-104.
20. Mangathayaru K. Pharmacognosy: an Indian perspective. Pearson Education India, 2013.
21. Nayak BK, Lazar J. Antimicrobial efficacy of leaf extract of Cayratia pedata Lam., Vitaceae. International Journal of ChemTech Research. 2014; 6(14):5721-5725.
22. Rice-Evans C, Miller N, Paganga G. Antioxidant properties of phenolic compounds. Trends in plant science. 1997; 2(4):152-159.
23. Selvarani K, Bai GVS. Anti-arthritic activity of Cayratia pedata leaf extract in Freund's adjuvant induced arthritic rats. Int. J Res. Plant Sci. 2014; 4(2):55-59.
24. Smith PE, Krohn R, Hermanson GT, Mallia AK, Gartner FH, Provenzano M *et al*. Measurement of protein using bicinchoninic acid. Analytical biochemistry. 1985; 150(1):76-85.
25. Sreevidya N, Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. Journal of AOAC international. 2003; 86(6):1124-1127.
26. Stanley AL, Ramani VA, Ramachandran A. Phytochemical Screening and GC-MS Studies on the Ethanolic Extract of Cayratia pedata. International Journal of Pharmaceutical and Phytopharmacological Research. 2012; 1(3):112-116.
27. Rajendran V, Indumathy S, Gopal V. Anti-nociceptive activity of Cayratia pedata in experimental animal models. Journal of Pharmacy Research. 2011; 4(3):852-853.
28. Rajendran V, Rathinambal V, Gopal V. A preliminary study on antiinflammatory activity of Cayratia pedata leaves on Wister albino rats. Der Pharmacia Lettre. 2011; 3(2):433-437.
29. Wang Y, Yang X, Zheng X, Li J, Ye C, Song X. Theacrine, a purine alkaloid with anti-inflammatory and analgesic activities. Fitoterapia. 2010; 81(6):627-631.
30. You Q, Chen F, Wang X, Jiang Y, Lin S. Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase. LWT-Food science and technology. 2012; 46(1):164-168.