

Phytochemical investigation and diuretic activity of *Cyclea peltata* leaf extracts

K. K. Hullatti, U. V. Gopikrishna¹,
I. J. Kuppast²

Department of Pharmacognosy,
KLE University College of Pharmacy,
Belgaum, ¹Departments of
Pharmacognosy, and ²Pharmacology,
National College of Pharmacy,
Shimoga, India

J. Adv. Pharm. Tech. Res.

ABSTRACT

Ayurvedic system of medicine is well known for treating renal problems. A vast number of medicinal plants mentioned in Ayurvedic system of medicine are known to possess diuretic properties. Present study reports the preliminary phytochemical investigation of petroleum ether and ethanolic extracts of *Cyclea peltata* and their diuretic activity. Preliminary phytochemical screening reveals the presence of phytosterols and alkaloids as major phytoconstituents in petroleum ether extract. The ethanolic extract showed the presence of alkaloids, flavonoids, tannins, diterpenes and saponins. Pharmacological investigation revealed that ethanolic extract of *C. peltata* leaves possessed significant diuretic activity in a given dose of 200 and 300 mg/kg body weight (Diuretic action 1.7 and 2.6, respectively). Where as petroleum ether extract has shown moderate diuresis at a dose of 300 mg/kg body weight (Diuretic action 1.1). The present study justifies the use of *C. peltata* in the Ayurvedic system of medicine as a diuretic drug.

Key words: *Cyclea peltata*, diuretic, furosemide, phytochemical

INTRODUCTION

Cyclea peltata (Lam) Hook. f. Thoms (Fam: Menispermaceae) is a common plant in the west coast of India. The roots of *C. peltata* are being used in Ayurvedic system of medicine under the name Rajapatha for various medicinal properties.^[1,2] The leaves of *C. peltata* were being used traditionally as coolant, antidandruff, antipyretic and diuretic.^[3,4] Traditionally leaves of *C. peltata* are being used for the treatment of herpes in some parts of costal Karnataka.^[5] The leaves of *C. peltata* are found to contain alkaloids such as cycleanine, bebeerines, hayatinin, hayatidin and hayatin. Five bisbenzylisoquinoline alkaloids, cycleapeltine, cycleadrine, cycleacuine, cycleanorine and

cycleahomine chloride have been isolated from the roots of *C. peltata*.^[6] The protective effect of *C. peltata* leaf on cisplatin-induced nephrotoxicity and oxidative damage has been reported. The results Indicated that the post-treatment of *C. peltata* leaf extracts might effectively ameliorate the oxidative stress parameters observed in cisplatin-induced renal toxicity and could be used as a natural antioxidant against cisplatin-induced oxidative stress.^[7]

The present study reports the phytochemical investigation and diuretic activity of petroleum ether and ethanolic extracts of *C. peltata* leaves.

MATERIALS AND METHODS

Plant Material

The plant *C. peltata* was collected from local area of Shimoga, Karnataka. The collected material was authenticated by Prof. D. Ruddrappa, Head, Department of Botany, Sahyadri Science College, Shimoga (NCP/COG/002/2008). The leaves were cleaned, air-dried, coarsely powdered, and subjected for Soxhlet-extraction by using petroleum ether and ethanol successively. The extracts were evaporated to dryness by using Rotary-evaporator under reduced pressure at 45°C.

Preliminary Phytochemical Investigation

Both the extracts were subjected to preliminary phytochemical screening for the determination of major chemical groups by standard procedures.

Address for correspondence:

Dr. Kirankumar Hullatti,
Associate Professor, Department of Pharmacognosy,
KLE University's College of Pharmacy, JNMC Campus,
Nehru Nagar, Belgaum - 590 010, Karnataka, India.
E-mail: khullatti@gmail.com

Access this article online

Quick Response Code:



Website:

www.japtr.org

DOI:

10.4103/2231-4040.90880

Thin Layer Chromatography

Plant extracts were dissolved in proper solvent and applied to precoated Thin Layer Chromatography (TLC) silica gel plates (silica gel 60 F₂₅₄, AluGram, Germany). Chromatograms were developed in solvent system containing Toluene: Ethyl acetate: Methanol (4: 0.5: 0.5) and the chromatograms were examined under UV and daylight as well as after derivetization with Anisaldehyde-Sulfuric acid and Dragendorff's reagent to detect the presence of different phytoconstituents.^[8]

Experimental Animals

The Institutional Animals Ethics Committee approved the use of animals for the present study. (Ethical clearance No. NCP/IAEC/CLEAR/P.COL/01/07/2007-08).

Wister albino rats of either sex weighing 200-300 g and mice weighing between 20 and 25 g were used for the experiment work. Animals were maintained under standard conditions husbandry, room temperature $26 \pm 2^\circ\text{C}$, relative humidity of 45-55%, 12 h light/dark cycle, in an animal house approved by the committee for the purpose of control and supervision of experiments on animals. Animals were obtained from the central animal house, national college of pharmacy, Shimoga. The animals were given standard diet supplied by Pranav Agro Industries Ltd., Sangli. The animals had free access of standard diet and water and housed in a poly propylene cages.

Acute Toxicity Study

The acute toxicity of petroleum ether and ethanolic leaf extracts of plant *C. peltata* was determined using Swiss albino mice. The animals were divided in to three different groups. The group 1 received normal saline (25 mL/kg), served as control. The group 2 and 3 received 3000 mg/kg body weight of petroleum ether and ethanolic extracts of leaves of *Cyclea peltata*. After oral administration of these extracts, the animals were observed continuously for the behavioral changes for the first 2, 4 h and then observed for mortality if any, after 24 h.^[9]

Evaluation of Diuretic Activity

Diuretic activity was evaluated on petroleum ether and ethanolic extracts of leaves of plant of *C. peltata* using Lipschitz *et al.* method.^[10,11] Healthy Wister rats of either sex were divided in to six groups of six animals each. Furosemide (20 mg/kg) was used as standard reference drug. All the drugs were prepared by suspending in 0.5% w/v of Trgacanth mucilage. Before the experiment, the rats were fasted for 18 h with free access to water. On the day of experiment, the animals of group 1 was administered with saline orally (2.5 mL of 0.9% NaCl/100 g body weight),^[12] this group served as control. Group 2 was treated with standard drug Furosemide (20 mg/kg) along with saline solution. Group 3 and 4 received petroleum ether extracts of *C. peltata* 200 mg/kg body weight and 300 mg/kg body weight along with saline solution, respectively. The group

5 and group 6 received orally ethanol extracts of *C. peltata* 200 mg/kg body weight and 300 mg/kg body weight with saline solution, respectively. Immediately after the treatment, the animals were placed in metabolic cage (1 animal in one metabolic cage) provided with wire mesh bottom and a funnel to collect the urine. Stainless steel sieves are placed in the funnel to retain fecal matter and to allow the urine to pass. The urine was collected in measuring cylinder up to 5 h for all control and drug-treated groups. During this period no food or water was made available to the animals. The volume of urine, electrolytes (Na^+ , K^+ , Cl^-) were estimated in the urine for assessment of diuretic activity. Na^+ , K^+ estimation was carried out using flame photometry.^[13,14] The Cl^- ion concentration was estimated by titration with 0.02 N AgNO_3 using 5% potassium chromate solution as indicator.^[15] The volume of urine was estimated for the assessment of diuretic activity. The diuretic action of tested drug was calculated by using the following formula:

$$\text{Diuretic action} = \frac{\text{Urinary excretion of test drug}}{\text{Urinary excretion of control}}$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of the test drug}}{\text{Diuretic action of Furosemide}}$$

Statistical Analysis

The values were expressed as mean \pm SEM. The statistical analysis was carried out by one-way analysis of variance (ANOVA) using ezANOVA v. 0.97 software. *P*-values < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical screening reveals the presence of phytosterols and alkaloids in petroleum ether extracts. The ethanolic extracts showed the presence of alkaloids, flavonoids, tannins, diterpenes and saponins.

Optimization of TLC Solvent System for the Extract

The extract was dissolved in methanol and subjected for TLC analysis. The solvent system which showed maximum separation of phytochemicals was Toluene: Ethyl acetate: Methanol (4: 0.5: 0.5). About 9 spots in visible light, 10 spots in UV long wave length and 7 spots in UV short wavelength had been detected [Figure 1]. The TLC plate was sprayed with Anisaldehyde-Sulphuric acid reagent and about 9 spots were been detected [Figure 2].

The total alkaloid isolated from the ethanolic leaf extract subjected for TLC analysis. The total alkaloid fraction was dissolved in methanol, and TLC plate was developed and the spots were detected by spraying the TLC plate with Dragendorff's reagent. The solvent system which showed maximum separation of alkaloids was Cyclohexane: Diethylamine (3: 0.5), which showed 3 spots [Figure 3].

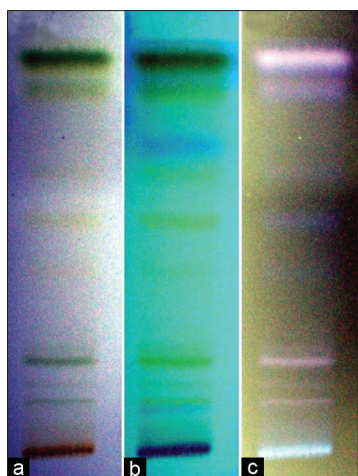


Figure 1: (a) Thin layer chromatography (TLC) of leaf ethanolic extract in visible light. (b) TLC of leaf ethanolic in UV-Long wavelength. (c) TLC of leaf ethanolic extract in UV-short wavelength

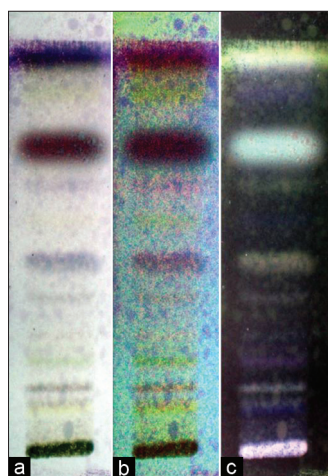


Figure 2: (a) TLC of leaf ethanolic extract in visible light after spraying with anisaldehyde-sulphuric acid reagent. (b) TLC of leaf ethanolic extract in UV-Long wavelength after spraying with anisaldehyde-sulphuric acid reagent. (c) TLC of leaf ethanolic extract in UV-short wavelength after spraying with anisaldehyde-sulfuric acid reagent



Figure 3: TLC of leaf ethanolic extract in visible light after spraying with Dragendorff reagent

Acute Toxicity Study

Leaf extracts of *C. peltata* were found to be safe orally at the dose of 3000 mg/kg body weight. No mortality was seen at this dose in any of the treated animals after 24 hours. So, the effective dose was chosen 200 and 300 mg/kg body weight.

Evaluation of Diuretic Activity

Treatment with the dose level of 200 and 300 mg/kg body weight of each of petroleum ether and ethanolic extracts of *C. peltata* produced significant diuresis in the treated animals. The ethanolic extract showed significant diuretic activity in dose-dependent manner compared to control rats at 5 h after the dose. Significant diuretic activity was observed with ethanolic extracts of *C. peltata* with both 200 and 300 mg/kg body weight (urine volume of 6.49 and 9.73 mL/kg body weight, respectively) compared to petroleum ether extract (urine volume of 3.65 and 3.93 mL/kg body weight, respectively). Urine output continues to be stimulated throughout the study period, such as cumulative urinary excretion was significantly higher in ethanolic extract compared to petroleum ether extract. The ethanolic extract of *C. peltata* showed more rapid and higher excretion of urine than the petroleum ether extract. The effect became significantly higher at 1 h after the dose administration compared to control rats ($P < 0.01$). The diuretic effect remained significantly higher for ethanolic extract treated rats as compared to control rats at all time points, such that the cumulative 5 hour urine excretion for ethanolic extract treated rats was more significant compared to control rats ($P < 0.01$). The ethanolic extract of *C. peltata* showed higher excretion of urine at 300 mg/kg body weight dose among all the tested extracts. The results are compiled in [Table 1] and [Figure 4].

Effect on Electrolytes

The urinary level of Na^+ was significantly increased in all the doses of both ethanolic and petroleum ether extracts of *C. peltata*. Significant increase in K^+ level in the extracts treated groups as compared to control animals was observed. K^+ level were higher in ethanolic extract treated groups compared to the petroleum extract treated groups. A dose-dependent increase in the both Na^+ and K^+ levels in urine was observed. None of the extracts including standard had shown much changes in Cl^- level. Results are given in the [Table 1].

SUMMARY AND CONCLUSIONS

The petroleum ether and ethanolic extract of *Cyclea peltata* were tested for their diuretic activity. From the present investigation we can conclude that, both the petroleum ether and ethanolic leaf extracts of *Cyclea peltata* has shown diuretic action, which is significant with ethanolic extract compared to petroleum ether leaf extract of *Cyclea peltata*. However, the phytoconstituents(s) responsible for this activity need to be investigated. Based on the pattern of excretion of water, Na^+ and K^+ , it appears that

Table 1: Effect of leaf extracts of *Cyclea peltata* on urine excretion and ionic concentration in rats

Treatment	Dose	Vol. urine (mL/kg b.w)	Diuretic action	Electrolyte concentration (mmol/l)			
				Na ⁺	K ⁺	Cl ⁻	Na ⁺ /K ⁺
Saline	25 mL/kg	3.68 ± 0.33	1.0	88.70 ± 0.95	79.05 ± 2.25	83.40 ± 2.01	1.1
Furosemide	20 mg/kg	15.26 ± 0.58**	4.1	129.20 ± 2.26**	89.73 ± 1.79**	83.75 ± 1.19	1.4
CPPE	200 mg/kg	3.65 ± 0.19	1.0	91.30 ± 2.65*	82.21 ± 0.85*	83.10 ± 2.15	1.1
	300 mg/kg	3.93 ± 0.29	1.1	94.20 ± 2.28**	83.48 ± 2.70*	82.20 ± 2.87	1.1
CPEE	200 mg/kg	6.49 ± 0.56**	1.7	107.80 ± 3.37**	85.52 ± 1.98**	83.55 ± 0.18	1.2
	300 mg/kg	9.73 ± 0.67**	2.6	121.30 ± 3.39**	87.60 ± 2.98**	83.35 ± 1.05	1.3

Each value represents mean ± S.E.M. of six rats. *P<0.05, **P<0.01. (The statistical analysis was carried out by one way analysis of variance using ezANOVA v. 0.97 software.) CPPE: *Cyclea peltata* Petroleum ether extract; CPEE: *Cyclea peltata* ethanolic extract

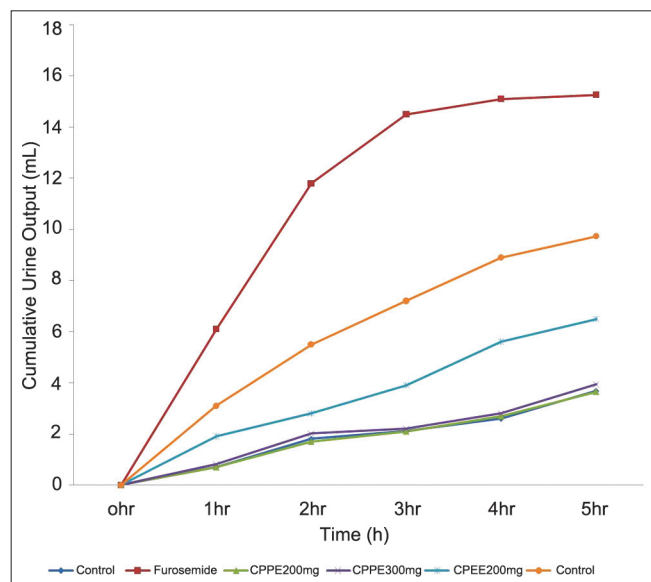


Figure 4: Time course of diuresis in rats with leaf extracts of *Cyclea peltata*, vehicle and furosemide

the active principle present in these two extracts having a Furosemide like activity. Some of the plants with potent diuretic and hypotensive activity were found contain benzyl isoquinoline type of the alkaloids.^[16] Certain flavonoids were found to exert their diuretic activity by binding with Adenosine A1 Receptor associated with the diuretic action.^[17] The diuretic activity of studied plant may be through any of these possible mechanisms since it is rich in alkaloids and flavonoids. The precise site, molecule and cellular mechanisms of the extract remain to be elucidated. The present study supports the traditional use of the plant *C. peltata* for its diuretic activity. Hence, there is a further scope in detail phytochemical investigation and activity guided isolation of active constituents from the plant *C. peltata*. The parameters such as phytochemical evaluation and the TLC system will help in further standardization of *C. peltata* in future.

REFERENCES

- Prajapati ND. Agro's dictionary of medicinal plants. Agrobios (India). New Delhi: Eastern Book Corporation; 2005. p.100.
- Saldanha CJ. Flora of Karnataka. New Delhi: Oxford and IBH

Publishing Co; 1992. p. 94-100.

- Kingston C, Nisha BS, Kiruba S, Jeeva S. Ethno medicinal plants used by indigenous community in traditional healthcare system. Ethnobotanical Leaflets 2007;11:32-7.
- Bhandary MJ, Chandrashekar KR, Kaveriappa KM. Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. J Ethnopharmacol 1995;47:149-58.
- Bhandary MJ, Chandrashekar KR. Herbal therapy for herpes in the ethno-medicine of Coastal Karnataka. Indian J Tradit Knowl 2011;10:528-32.
- Kupchan SM, Liepa AJ, Baxter RL, Hintz HP. New and related artifacts from *Cyclea peltata*, J Org Chem 1978;38:1846-7.
- Vijayan FP, Rani VK, Vineesh VR, Sudha KS, Michael MM, Padikkala J. Protective effect of *Cyclea peltata* on Cisplatin induced nephrotoxicity and oxidative damage. J Basic Clin Physiol Pharmacol 2007;50:812-4.
- Wagner H, Blodt S. Plant drug analysis- A Thin Layer Chromatography Atlas, 2nd ed. Springer-Verlag, Berlin Heidelberg; 1996.
- Turner RA. The organization of screening in: Screening method in pharmacology Vol. 1, New York and London: Academic Press; 1965. p. 21.
- Mukharjee PK. Pharmacological screening of herbal drugs in: Quality control of herbal drugs, 1st ed. New Delhi: Business horizon pharmaceutical publisher; 2002. p. 537.
- Vogel HG. Drug discovery and evaluation; Pharmacological assays, 2nd ed. New York; Springer-Verlag, Berlin Heidelberg; 2002.
- Murari Y, Kokubugata G, Yokota M, Kitazima J, Iwashin T. Flavonoids and anthocyanins from six *Cassipoupa tax* (Lauraceae) as taxonomic markers, Biochemical's systematic and ecology, Biochemical Systematics and Ecology 2008;36:745-8.
- Jeffery GH, Basset J, Mundan J, Denny R. Vogel's Textbook of Quantitative Chemical Analysis. 5th ed. England: Addison Wesley Longman Ltd; 1989. p. 801.
- Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. Screening of Indian plants for biological activity, II. Indian J Exp Biol 1969;7:250-62.
- Beckett AH, Stanlake JB. Practical pharmaceutical chemistry. 1st ed. New Delhi: CBS Publishers and Distributor; 1997. p. 197.
- Erdemgil FZ, Baser KH, Kirimer N. Recent studies on the alkaloids of *thalictrum* species. Acta Pharmaceutica Turcica 2001;43:185-8.
- Yuliana ND, Khatib A, Link-Struensee AM, Ijzerman AP, Rungkat-Zakaria F, Choi YH, et al. Adenosine A1 receptor binding activity of methoxy flavonoids from orthosiphon stamineus. Planta Med 2009;75:132-6.

How to cite this article: Hullatti KK, Gopikrishna UV, Kuppast IJ. Phytochemical investigation and diuretic activity of *Cyclea peltata* leaf extracts. J Adv Pharm Tech Res 2011;2:241-4.

Source of Support: Nil, **Conflict of Interest:** Nil.