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Physio- Phytochemical screening and Diuretic activity of leaves of *Pavetta indica* Linn

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Abstract:

Pavetta indica Linn is used in the traditional medicine for many diseases.In the present study, the diuretic activity of Petroleum ether and Methanol extract of leaves of *Pavetta indica* was studied and the activity was compared with furosemide as standard.All the extracts exhibited significant diuretic activity as evidenced by increased total urine volume and the urine concentration of Na^+, K^+ and Cl^-. The results thus support the use of *Pavetta indica* as diuretic agent. The preliminary phyto chemical investigations were also be studied.

Keywords: *Pavetta indica*, Phytochemical Screening, Fluorescence analysis, Diuretic activity, Flavonoids.

Introduction:

Pavetta indica linn. is available at the greater part of India ascending to an altitude of 1500 m in the Himalayas; it has also recorded from the Andaman. It belongs to the family *Rubiaceae*. A shout bushy shrub 0.6-1.2 m high; bark thin, smooth, yellowish; young branches terete, glabrous. Leaves 7.5-15 by 2.5-6.3 cm, membranous, variable in shape and size, elliptic - oblong or elliptic - lanceolate, sometimes obovate - oblong, obtuse, acute or acuminate, glabrous on both sides, base tapering; main nerves 8-10 pairs; petioles 6-13 mm long; stipules connate, triangular, acute, thin, deciduous. Flowers white, odourous, in terminal sessile corymbose pubescent cymes; pedicles 4-6 mm long, densely pubescent; bracts broad, membranous, the lower copular; buds oblong- clavate. Calys densely pubescent, 3mm long; tube narrowly campanulate; teeth 1.25 mm long, triangular, acute, slightly reflexed at the tip. Corolla - tube 13 mm long; lobes 6-8 by 2.5 mm, linear - oblong, subacute. Style white, glabrous or nearly so; stigma green, narrowly clavate, puberulous. Fruit 6-14 mm diameter, glabose, black, smooth. The leaves and roots are employed in the preparation of poultices for boils and itches; decoctions of leaves are used as a lotion for ulcerated nose and for heamorrhoids. Root is used for anticephalagic. Leaf is used in haemorrhoidol pain and ulcerated nose. Wood is used as antirheumatic. Fruits are used as anthelmintic [1,2,3,4,5].

Materials and Methods:

The plants of *pavetta indica* linn were collected from Madurai during the months of June and identified by Dr. Stephen (Professor, American college Madurai). The plants were then washed with water to remove soil and other extraneous matter. The leaves of plant were cut into small pieces and were dried under shade for 20 days. Then the dried material was homogenized to coarse powder and was stored in airtight container.

Preparation of the Extract

About 400 gms of dry coarse powder was soaked with petroleum ether (2500ml) for two days. After this, soaked materials were extracted with petroleum ether (40˚C-60˚C) by continuous hot percolation method for 72 hrs. The petroleum ether extracts were filtered and concentrated under reduced pressure. A green- black residue was obtained (15 gms). The mark left after the petroleum ether extraction then dried and extracted with methanol (2600 ml) for 72 hrs. The methanolic extract were also filtered and concentrated under reduced pressure. A dark black residue was obtained (15 gms). Crude extracts were stored in desiccators.

Physico- Chemical standards [6,7,8]

Physico- chemical parameters of the powdered drug such as ash value, extractive value, loss on drying were performed according to the method. Extracts were prepared by various solvents by standard methods and percentage of dry extract was calculated in terms of air-dried leaf powder. (Table 1, 2, 3)

Table 1: Ash values

S. no	Type of ash	Results
1.	Total ash	14.21 % w/w
2.	Acid insoluble ash	0.84 % w/w
3.	Water soluble ash	2.22 % w/w

Table 2: Extractive value, Percentage yield and colour of extracts

Solvent used	Percentage yield	Colour of extract
Petroleum ether	1.2	Green
Methanol	9.2	Blackish green

Table 3: Loss on drying

Loss on drying	7.53%

Table 4: Preliminary phytochemical screening of the pet.ether & methanol extract of pavetta indica linn.

S. No	Phytochemicals	Petroleum ether extract	Metha-nolic extract
1	Carbohydrate	(+) ve	(+) ve
2	Glycosides	(+) ve	(+) ve
3	Phytosterol	(+) ve	(+) ve
4	Saponins	(+) ve	(+) ve
5	Fixed oil & fats	(-) ve	(-) ve
6	Tannins	(-) ve	(-) ve
7	Proteins & amino acids	(-) ve	(-) ve
8	Flavanoids	(-) ve	(+) ve
9	Alkaloids	(-) ve	(+) ve
10	Coumarins	(-) ve	(-) ve

(+) ve - indicates positive test result, (-) ve - indicates negative test result

Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study [9,10] (Table 4)

Behaviour of leaf powder with different chemical reagents

Behaviour of leaf of ***Paveeta indica*** with different chemical reagents was performed to detect the occurrence of phytoconstituents along with colour changes under ordinary daylight by standard method [11] (Table 5)

Determination of Saponin

According to the results obtained from positive foaming test and high foaming index [12] of leafs of ***Paveeta indica*** study was carried out for the estimation of total saponin content [13,14] (Table 6).

Preliminary phytochemical investigation

The qualitative chemical test of various extracts of ***Paveeta indica*** was carried out using standard procedure. Glycosides, Phytosterols, Saponins, Flavonoids and Alkaloids are present in petroleum ether and methanol extracts [15,16,17,18].

Thin Layer Chromatography

About 30gms of silica gel – B was weighed out and it was shaken with 100ml of water to form a homogenous suspension. The suspension was poured into a thin layer chromatography applicator which was adjusted to 0.25mm thickness. 20 to 40 Carrier plates (20.5cm) were laid down for air drying. The plates were kept in the hot air oven at 110°C for one hour to activate the silica gel – G. The plates were stirred in a dry atmosphere and used whenever required. By using the capillary tube the extracts are spotted on the T.L.C plates 2cm above the bottom and in the chromatogram in various solvent systems for different compounds. The spots are developed in solvent system were identified by means of different spraying reagents.

$$R_f \text{ value} = \frac{\textit{distance travelled by solute}}{\textit{distance tavelled bysolvent}}$$

Table 5: Fluorescence characteristics of leaf extract of *Paveeta indica*

Powder + Reagent	Color observed in Ordinary light	Color observed under Ultra violet light Short (254 nm)	Color observed under Ultra violet light Long (365 nm)
Powder	Brown	Green	Green
Powder+ 1N NaOH in methanol	Greenish black	Green	Green
Powder+ 1 N NaOH in water	Brownish green	Green	Black
Powder++ 1 N HCl	Brownish yellow	Green	Black
Powder+50% HNO_3	-	Light Green	Black
Powder+50% H_2SO_4	Slight brown	Green	Black
Powder+Methanolic NaOH.dried+ nitrocellulose in aceticacid	Yellowish green	Dark Green	Black
Powder+ 1N NaOH + nitrocellulose in aceticacid	Dark brown	Light Green	Greenish Black

Evaluation of Diuretic activity

Male rats (wister albino strain) weighing 150 to 180gm were maintained under standard condition of temperature and humidity. They were fed with standard rat feed and water *adlibitum*. The method of Lipschitz *et al.*, [19,20] was employed for the assessment of diuretic activity. The experimental protocols have been approved by the Institutional Animal Ethical Committee. Four groups of six rats in each and were fasted and deprived of water for eighteen hours prior to the experiment. The first group of animals serving as control, received normal saline (25ml/Kg,p.o.); the second group received furosemide (20mg/Kg,i.p.) in saline; the third and fourth groups received the petroleum ether and methanol extracts at the doses of 250 mg/Kg, respectively, in normal saline. Immediately after administration the animals were placed in metabolic cages (2 per cage), specially desingned to separate urine and feaces, kept at room temperature of 25± 0.5ºC through out the experiment. The urine was collected in measuring cylinders up to 5hrs after dosing. During this period, no food or water was made available to animals. The parameters taken for individual rat were body weight before and after test period, total concentration of Na^+, K^+ and Cl^- in the urine. Na^+, K^+concentrations were measured by Flame photometry [21] and Cl^- concentration was estimated by titration with silver nitrate solution (N/50) [22]using three drop of 5% potassium chromate solution as indicator. Furosemide sodium salt was given by stomach tube. Optimal dose activity relation was found to be 20mg/Kg of furosemide per kg body weight in series of supportive experiments. Results are reported as mean ± SD, the test of significance ($p<0.01$) was stastically.

Statistical analysis

Data are reported as the mean ± SD of three measurements. Statistical analysis was performed by students' t test .

Result and Discussion:

Physio- Chemical standards

The percentage of loss on drying, total ash, acid insoluble ash ,water soluble ash, extractive values and colour of extracts are obtained by employing standard method of analysis and described in Table 1,2 and 3.The loss on drying is 7.53%. The total ash content is 14.21%, the acid insoluble ash content is 0.84% and water soluble ash

Reagent	Colour / ppt	Constitituent
Powder	Green	-
Powder + con. H_2So_4	No brown colour	Carbohydrate absent
Powder + aqueous $Fecl_3$	No Bluish black colour	Tannin absent
Powder + Iodine solution	No black	Starch absent
Powder + Aqs. $Hgcl_2$	Blue colour is produced	Alkaloids present
Powder + picric acid	Yellow colour is formed	Alkaloids present
Powder + Mg HCl	Mango colour is produced	Flavonoids present
Powder + aqueous $AgNo_3$	Precipitate is not formed	Protein absent
Powder + ammonia solution	Pink colour	Cardiac glycoside present
Powder + Aqs. KOH	Pink colour	Cardiac glycoside present
Powder + Aqs. Na nitrite	Red colour	Phytosterols present
Powder + Water (shaking)	Foam is produced	Saponins present

content is 2.22%.The percentage yield of petroleum ether and methanol extract is 1.2 % and 9.2 %.the colour of the extract is green and blackish green.

Table 6: Behaviour of leaf extract of *Paveeta indica*

Preliminary phytochemical investigation

Preliminary phytochemical screening of the ***Paveeta indica*** plant powder was done per standard methods and results are presented in the Table 4.

Table 7: Results of Quantitative estimation of leaf extracts of *Paveeta indica*

S. No	Estimation		Results
1.	Foaming index		More than 1000
2.	Total saponin content	Method I Method II	9.5% w/w 10.4 % w/w

Petroleum ether extract shows the presence of carbohydrate, glycoside, phytosterols and saponin. Methanol extract shows the presence of carbohydrate, glycoside, phytosterols, saponin , flavanoids and alkaloids.In both the extracts fixed oil ,fat, tannins and coumarin are absent. The medicinal properties exhibited by this species are due to the presence of alkaloids, flavanoids and glycosides.

Fluorescence characteristics

Paveeta indica leaf extract powder treated with1N NaOH in methanol shows black colur in ordinary light and green colour in 254 and 365 nm. In 1N NaOH in water greenish black in visible and green colour in 254 and 365 nm. In 1N HCl it shows blackish yellow in visible light and green, black colour in 254 and 365 nm. In 50% HNO_3 it does not show any colour in ordinary light and light green , black colour in 254 and 365 nm. In 50% H_2SO_4 it shows slight brown colour in visible light and green, black colour in 254 and 365 nm. In methanolic NaOH + dried nitro cellulose in acetic acid it shows yellowish green in visible light and dark green, black colour in 254 and 365 nm. In 1N NaOH + dried nitro cellulose in acetic acid it shows dark brown in visible light and light green , greenish black colour in 254 and 365 nm. The result of fluorescence analysis is shown in the Table 5.

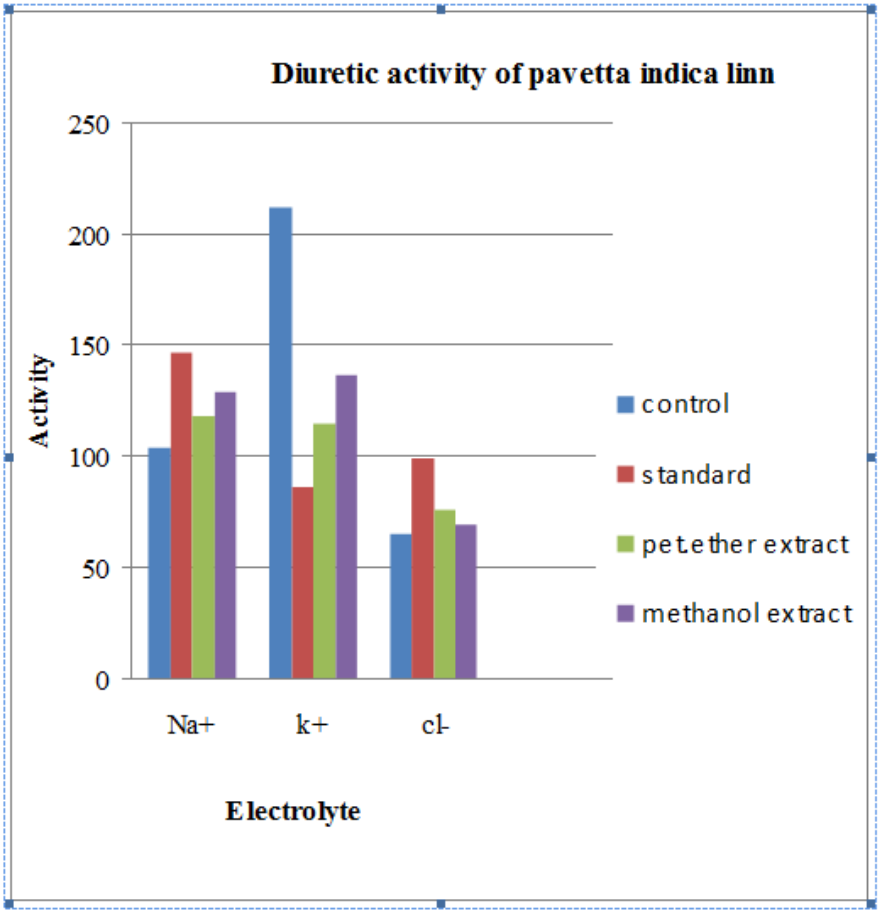
Behaviour of leaf powder with different chemical reagents

Paveeta indica leaf powder treated with Con H_2SO_4 it shows no blue colour.

Table 9: Diuretic activity of petroleum ether and methanol extracts of leaves of *Pavetta indica linn.*

S.no.	Treatment	Dose	Urine volume (ml) 24 hr.	Electrolyte excretion			
				Na^+	K^+	Cl^-	Na^+/K^+
1	Control	25ml/kg 1%CMC	6.9±1.02	104.2± 3 .42	212.2± 9.02	65.2± 3.12	0.49± 0.37
2	Standard (Frusemide)	25mg/kg I.P.	14.2± 2.02 bb(a)	146.4± 4.10 bb(a)	86.4± 4.02 bb(a)	98.9± 3.06 bb(a)	1.69± 1.01
3	Pet.Ether extract	250mg/kg Suspension With1%CMC	7.9±1.01	118.4± 4.08	114.6± 4.26	76.4± 2.96	1.03± 0..95
4	Methanol extract	250mg/kg Suspension With1%CMC	8.8±1.18 bb(b)	129.3± 5.21 bb(b)	136.5± 5.62 bb(b)	69.3± 2.06 bb(b)	0.94± 0.92 bb(b)

- Values are expressed as Mean ± SEM
- Values are find out by using ANOVA followed by Newman level's multiple range tests.
- bb(a) values were significantly different from control at (P<0.01)
  bb(b) values were significantly different from standard at (P<0.01)



Aqueous $FeCl_3$ it gives no bluish black colour. Leaf powder treated with iodine it gives no blue colour. Aqeous mercuric chloride it gives blue colour. Powder is treated with picric acid it gives yellow colour. Magnesium hydrochloride it gives mango colour. Powder is treated with ammonia solution, Aqueous KOH, Aqueous $NaNo_3$ it gives pink colour. Powder is treated with water and shake it , foam is produced.

Determination of Saponin

The total saponin content is 9.5% and 10.4% and the foaming index is more than 1000.

Thin Layer Chromatography

For find out the active constituents in the leaf extract the thin layer chromatography is done. Glycosides the solvent system used is ethylacetate , pyridine and water (5:1:4) and spraying reagent is chloroform and orange colour is produced. Saponins solvent system used is butanol, water (1:1) and spraying reagent is Con HCl and dark brown colour is produced. For phytosterols solvent system used is hexane, ethylacetate (1:1) and spraying reagent used is stannic chloride and orange brown round is produced. For flavanoids butanol , acetic acid, water and ether (9:6:1:3) and spraying reagent used is phenol sulphuric acid and greenish black colour is produced. Alkaloids methanol , ammonium hydroxide (5:5) and spraying reagent dragendroff's reagent and orange brown colour is produced.

Evaluation of Diuretic activity

All these extracts at 250 mg/kg showed increase in urine volume and also the concentration of Na^+, K^+, Cl^- in urine (Table 8) may revealed in the specific ion responsible for the diuretic effect. Among these extracts significant diuretic activity was observed with methanolic extract of *pavetta indica*. Also methanol extract produced significant fall in K^+ excretion compared to control ($P<0.01$). Diuretics relive pulmonary congestion and peripheral edema. These agents are useful in reducing the syndrome of volume overload, including orthopnea and paroxysmal nocturnal dyspnoea. They decrease plasma volume and subsequently venous return to the heart (preload). This decreases cardiac workload, oxygen demand and plasma volume, thus decreasing blood pressure . Thus, diuretics play an important role in hypertensive patients. In present study, we can demonstrate that ethanol, aqueous and chloroform extract may produce diuretic effect by increasing the excretion of Sodium, Potassium and Chloride. The control of plasma sodium is important in the regulation of blood volume and pressure; the control of plasma potassium is required to maintain proper function of cardiac and skeletal muscles. The regulation of Sodium, Potassium balance is also intimately related to renal control of acid-base balance. The Potassium loss that occurs with many diuretics may lead to hypokalemia. For this reason, generally potassium-sparing diuretics are recommended . In present study Petroleum ether and methanol extracts showed elevated levels of Potassium in urine, which may increase risk of hypokalemia and hence its potassium sparing capacity has to be investigated. Active principles such as flavonoids, saponins, phytosterols and terpenoids are known to be responsible for diuretic activity [23,24,25] . These active principles in the extracts may be responsible for diuretic activity. It may be presumed that the diuretic activity due to the presence of flavanoids in the extract.

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