

# Comparative pharmacognosy of Pashanbhed

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## ABSTRACT

**Background:** *Pashanbhed* is a commercially available diuretic and lithotropic drug, used to treat renal problems. It is a controversial name as it is assigned to various plants such as *Bergenia ligulata*, *Kalanchoe pinnata*, *Coleus aromaticus* and *Rotula aquatica*. **Objective:** To perform the comparative preliminary phytochemical screening, diuretic activity, and thin layer chromatography (TLC) finger printing profile of three plants (*B. ligulata*, *C. aromaticus*, and *K. pinnata*), most commonly used as *Pashanbhed*. **Materials and Methods:** Diuretic potential of methanolic extract (ME) of three plants were evaluated at two dose levels (500 and 1,000 mg/kg p.o.), using normal Wistar rats (Lipschitz method). Furosemide (20 mg/kg p.o.) was used as a standard drug. The effect on urine output and electrolyte changes were measured for 24 h and compared. All MEs were screened preliminarily for their constituents and their TLC finger printing profiles were prepared. One-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.  $P < 0.05$  was considered statistically significant. **Results:** The MEs of all three plants have shown diuresis in normal rats. However, in intercomparison of the ME *C. aromaticus* (1,000 mg/kg p.o.) produced more significant diuresis ( $P < 0.05$ ) and electrolyte excretion compared to other test groups, the effect was at par with furosemide. The ME of these plants showed presence of alkaloids, glycosides, steroids, terpenoids, saponins, flavonoids, etc. **Conclusion:** The ME of *C. aromaticus* (1,000 mg/kg p.o.) has showed highest diuretic action (4.2) among the tested extracts. This suggests the use of *C. aromaticus* leaves as "*Pashanbhed*"; the most effective diuretic drug.

**Key words:** *Bergenia ligulata*, *coleus aromaticus*, diuretic, *kalanchoe pinnata*, *pashanbhed*

## INTRODUCTION

Diuretics are the drugs capable of increasing the rate of urine flow and sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations including hypertension, heart failure, renal failure, nephritic syndrome, and cirrhosis. These drugs act on the kidney and are able to increase the volume of urine excretion.<sup>[1]</sup> *Pashanbhed* (meaning 'stone breaker') is a well-known Ayurvedic drug, used mainly as diuretic and

lithotropic. It is a controversial drug, as plants like *Bergenia ligulata* Wall., *Aerva lanata* Juss., *Coleus aromaticus* Benth., *Kalanchoe pinnata* Linn., *Homonoia riparia* Lour., *Rotula aquatica* Lour., etc., are being used under the name *Pashanbhed*.<sup>[2]</sup> Most common species of marketed formulations used as *Pashanbhed* are *B. ligulata* (rhizomes), *C. aromaticus* (leaves), and *K. pinnata* (leaves). These species are found in most of the proprietary drugs for example, Calcure tablets, Nieren syrup and capsule, Urotone tablet (*B. ligulata*), Nefrol capsules and syrup, Kid clear capsule (*C. aromaticus*), and Parnabija svarasa (*K. pinnata*). Therefore, in the present study, these three plants were selected for the comparison of diuretic activity. Traditionally, *B. ligulata* (Family: Saxifragaceae) rhizomes are used for constipation, kidney stones, and urinary problems and has been tested for antiurolithic and diuretic property;<sup>[3,4]</sup> the leaves of *C. aromaticus* (Family: Lamiaceae) are used as expectorant, liver tonic, lithotropic, and diuretic<sup>[5]</sup> and has been investigated for diuresis.<sup>[6]</sup> Moreover, the leaves of *K. pinnata* (Family: Crassulaceae) are used in the traditional system of medicine for diabetes, diuresis, kidney stones, and respiratory tract infection<sup>[7]</sup> and also evaluated for antiurolithic property.<sup>[8]</sup>

The description about *Pashanbhed* as a diuretic drug is vague hence; three plants species (*B. ligulata*, *C. aromaticus*, and

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*K. pinnata*) have been used as source of this drug. Hence, present study has been designed to compare diuretic potential of these three plant materials to identify the most suitable plant source as *Pashanbhed*. Preliminary phytochemical screening and thin layer chromatography (TLC) finger printing profiling was done as a part of qualitative evaluation of these three plant extracts.

## MATERIALS AND METHODS

### Collection and identification of plant material

*B. ligulata* (rhizomes) and *K. pinnata* (leaves) were procured from VHCA, Herbals, Karnal and authenticated by Dr. HB Singh, Director, Department of Raw Material Herbarium and Museum, National Institute of Sciences Communication and Information Resources (NISCAIR), New Delhi (Ref. No. NISCAIR/RHMD/Consult/-2012-2013/110). *C. aromaticus* (leaves) were procured from Tirupati, Andhra Pradesh and authenticated by Dr. K Madhava Chetty, Asst Professor, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India (voucher specimen no. 965).

### Preparation of methanolic extracts

The leaves of *C. aromaticus* and *K. pinnata* and rhizomes of *B. ligulata* were cleaned, air-dried, and coarsely powdered. MEs (95%) of each plant (200 g) were prepared by soxhletion. The solvent was recovered under vacuum using rotary evaporator.

### Preliminary phytochemical screening

All three ME of selected plants were subjected to preliminary phytochemical screening to check the presence of various phytoconstituents following standard procedures.<sup>[9]</sup>

### TLC finger printing profile

TLC finger printing profiling of ME of all three plants have been prepared using high-performance thin layer chromatography (HPTLC) instrument (Camag, Switzerland) equipped with a sample applicator Linomat-5, TLC Scanner-3, Wincats 4.02, integration software. TLC precoated silica gel 60 F<sub>254</sub> aluminium plates (20 × 10) of 0.2 mm thickness, were used as a stationary phase. The mobile phase system comprised of toluene: ethyl acetate: acetone: formic acid (2:4:4:0.2 v/v/v), was used. The linear ascending development was carried out in a twin trough glass chamber. The optimized chamber saturation time for the mobile phase was 20 min at room temperature (25 ± 2°C) at a relative humidity of 50 ± 5%. The slit dimension of 6 × 0.45 mm and the scanning speed of 20 mm/s were employed. Chromatograms were examined under short ultraviolet (UV) radiation 254 nm, long UV (366 nm), and visible light after spraying with anisaldehyde-sulfuric acid reagent.<sup>[10,11]</sup>

## Screening of diuretic activity

### Animals

Male Wistar rats (220-250 g), three in each cage; were housed and maintained under standard laboratory conditions at 25 ± 2°C, normal 12 h:12 h light/dark schedule with free access to water and food. The animals were kept for 3 weeks to adapt to the laboratory environment before experimental procedure. All studies were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). (Approval letter No-IAEC/CPCSEA/M6/95).

### Drugs and chemicals

All chemicals used during the study were purchased from E-merck and of analytical grade; furosemide was purchased from Aventis Pharmaceuticals Ltd.

### Evaluation of diuretic activity

The method of Lipschitz *et al.*,<sup>[12,13]</sup> was employed for assessment of diuretic activity. In this method, male rats (220-250 g), deprived of food and water for 18 h prior to the experiment were divided into eight groups of six rats each. The first group of animals, serving as control, received normal saline (25 ml/kg, p.o.); the second group received furosemide (20 mg/kg, p.o.) in saline; the third, fourth, and fifth groups received the ME of *B. ligulata*, *C. aromaticus* and *K. pinnata* respectively, at the dose 500 mg/kg, p.o.; whereas the sixth, seventh, and eighth received the ME at the dose 1,000 mg/kg, p.o. in normal saline. The animals were individually placed in metabolic cages and kept under investigation for 24 hrs. All the urine produced in 24 h period was collected in a test tube through plastic pipe attached to a specially designed metabolic cage. During this period no food or water was made available to the animals. The volume and electrolyte concentration of urine were estimated for the assessment of diuretic activity. The sodium and potassium contents of urine were determined by flame photometry.<sup>[14,15]</sup> The chloride ion concentration was estimated by titration method.<sup>[16]</sup> The diuretic action of tested drug was calculated by using the following formula:<sup>[17]</sup>

Diuretic action

$$= \frac{\text{Urinary excretion of test drug treated animals}}{\text{Urinary excretion of control animals}}$$

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

### Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM). The difference between experimental groups was compared by one-way analysis of variance (ANOVA)

followed by Bonferroni's multiple comparison test. The results were considered statistically significant when  $P < 0.05$ .

## RESULTS

The yield of ME of *B. ligulata*, *C. aromaticus*, and *K. pinnata* was 13.66, 16.07, 22.00%w/w, respectively. Preliminary phytochemical screening of ME of all three plants showed the presence of carbohydrates, tannins, phenolic compounds, and flavonoids. In addition, ME of *B. ligulata* showed the presence of glycosides and saponins; *C. aromaticus* showed the presence of alkaloids, terpenoids, and saponins; and *K. pinnata* revealed the presence of steroids and glycosides.

### TLC finger printing profile

The TLC fingerprinting profile of *B. ligulata*, *K. pinnata*, and *C. aromaticus* had shown seven, five, and three spots at short UV [Figure 1a]; four, four, and three spots at long UV [Figure 1b]; and four, five, and three spots in visible light [Figure 1c]; respectively; with different  $R_f$  values [Table 1].

### Evaluation of diuretic activity

#### Effect on urine volume

The groups of animal treated with tested drugs have shown increase in urine volume at the dose levels of 500 and 1,000 mg/kg p.o. and diuretic action was more than 1. However, in inter-comparison, the *C. aromaticus* has shown more significant ( $P < 0.05$ ) diuretic action [Table 2].

#### Effect on electrolytes

The urinary level of electrolytes ( $\text{Na}^{++}$  and  $\text{K}^{+}$ ) was

significantly increased in all the animals treated with test drugs in comparison to control group. However, inter-group comparison has shown more significant increase in group of animals treated with *C. aromaticus* [Table 2].

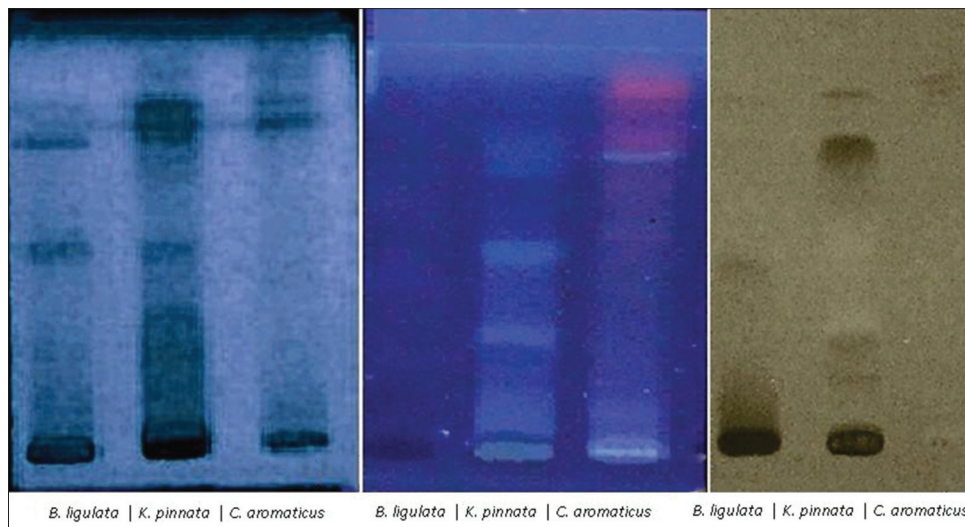
## DISCUSSION

Diuretics modulate the volume and composition of body fluids in variety of clinical conditions like hypertension, heart failure, nephritic syndrome, and cirrhosis. Herbal diuretics produce very little toxicity and the presence of rich potassium helps to prevent potassium depletion, thus giving the benefit of potassium sparing diuretic effect.<sup>[18]</sup> MEs of *B. ligulata*, *C. aromaticus*, and *K. pinnata* were tested for their diuretic activity. The results [Table 2], suggest that MEs of *B. ligulata*, *C. aromaticus*, and *K. pinnata* have dose-dependent diuretic action. The diuretic activity of MEs were found more ( $P < 0.05$ ) for all the selected plant materials at the dose 1,000 mg/kg p.o. as compared to 500 mg/kg p.o. The inter-group comparison of different test group animals suggests that *C. aromaticus* is the most suitable source of *Pashanbhed*. However, the phytoconstituents responsible for

**Table 1: Results of TLC finger printing profile for MEs of *Bergenia ligulata*, *Kalanchoe pinnata*, and *Coleus aromaticus* at short UV (254 nm), long UV (366 nm), and visible light**

Plant name	Rf at short UV (254 nm)	Rf at long UV (366 nm)	Rf at visible light
<i>B. ligulata</i>	0.06, 0.09, 0.34, 0.48, 0.53, 0.66, 0.86	0.03, 0.05, 0.56, 0.83	0.09, 0.46, 0.68, 0.87
<i>K. pinnata</i>	0.02, 0.07, 0.53, 0.71, 0.83	0.28, 0.50, 0.75, 0.89	0.06, 0.54, 0.72, 0.79, 0.84
<i>C. aromaticus</i>	0.07, 0.55, 0.75	0.07, 0.55, 0.77	0.07, 0.56, 0.75

ME=Methanol extract, TLC=Thin layer chromatography, UV=Ultraviolet



**Figure 1:** (a) TLC finger printing profiles of ME of *Bergenia ligulata*, *Kalanchoe pinnata*, and *Coleus aromaticus*, respectively, at short UV (254 nm). (b) TLC finger printing profiles of ME of *B. ligulata*, *K. pinnata* and *C. aromaticus*, respectively, at long UV (366 nm). (c) TLC finger printing profiles of ME of *B. ligulata*, *K. pinnata*, and *C. aromaticus*, respectively, at visible light



**Table 2: Effect of MEs of *Bergenia ligulata*, *Kalanchoe pinnata*, and *Coleus aromaticus* on urinary volume, sodium, potassium, and chloride concentration in Wistar rats**

Groups	Total urine volume/24 h (mL)	Diuretic action	Diuretic activity	Sodium content (meq/L)	Potassium content (meq/L)	Chloride content (meq/L)
Normal (0.9% saline)	2.6±0.35	1	0.1	57.86±0.73	11.63±0.40	58.27±0.35
Standard (20 mg/kg)	11.5±0.41*	4.4	1	87.37±0.66*	19.73±0.37*	96.84±0.36*
<i>B. ligulata</i> (500 mg/kg)	5.5±0.33* <sup>#^</sup>	2.1	0.5	64.08±1.53* <sup>#^</sup>	13.58±0.22* <sup>#^</sup>	64.23±0.36* <sup>#^</sup>
<i>B. ligulata</i> (1,000 mg/kg)	9.0±0.36* <sup>#^</sup>	3.5	0.8	71.45±0.60* <sup>#^</sup>	16.05±0.48* <sup>#^</sup>	75.32±0.71* <sup>#^</sup>
<i>C. aromaticus</i> (500 mg/kg)	7.2±0.38* <sup>#^</sup>	2.8	0.6	69.71±0.39* <sup>#^</sup>	14.93±0.36* <sup>#^</sup>	74.69±0.49* <sup>#^</sup>
<i>C. aromaticus</i> (1,000 mg/kg)	11.2±0.40*	4.3	0.9	83.43±1.33*	18.77±0.38*	94.88±0.29*
<i>K. pinnata</i> (500 mg/kg)	4.4±0.40* <sup>#^</sup>	1.7	0.4	63.28±0.50* <sup>#^</sup>	14.45±0.26* <sup>#^</sup>	62.81±0.59* <sup>#^</sup>
<i>K. pinnata</i> (1,000 mg/kg)	7.4±0.37* <sup>#^</sup>	2.8	0.7	70.18±0.55* <sup>#^</sup>	15.77±0.38* <sup>#^</sup>	73.55±0.61* <sup>#^</sup>

*n*=6 in each group; values are mean±SEM and *P*<0.001. \*Significant with respect to control, <sup>#</sup>significant with respect to standard, <sup>^</sup>significant with respect to *C. aromaticus* (1 g/kg). One-way ANOVA was used for analysis and Bonferroni's multiple comparison test was applied. ME=Methanol extract, SEM=Standard error of the mean, ANOVA=Analysis of variance

this activity need to be investigated. Based on the pattern of excretion of water, Na<sup>+</sup> and K<sup>+</sup>, it appears that the active principle(s) present in this extract of *C. aromaticus* having a furosemide like activity, thus act as loop diuretics. Patel *et al.*, proved that tannins and phenolic compounds are responsible for diuretic property.<sup>[19]</sup> However, according to Ahmad *et al.*, and Bhadoriya *et al.*, alkaloids are being assumed as predominating factor for diuresis.<sup>[20,21]</sup> Some of the plants with potent diuretic and anti-hypertensive activity were found to contain benzyl isoquinoline type of the alkaloids.<sup>[22]</sup> Flavonoids are found to exert their diuretic activity by binding with adenosine A1 receptor associated with the diuretic action.<sup>[23]</sup> Many reports have extensively shown that terpenoids exert significant cardiovascular effects.<sup>[24-26]</sup> It has also been reported that some classes of diterpenes showed significant systemic hypotensive and coronary vasodilatory effects accompanied by gradual decrease in heart rate.<sup>[27,28]</sup> Saponins are also known to have several physiologic activities, depending on their chemical structures, such as hemolytic properties, alteration of membrane permeability, particularly the modulation of renal sodium excretion.<sup>[29]</sup>

Thus, diuretic activity of *C. aromaticus* may be through synergistic mechanisms since it is rich in alkaloids, phenolic compounds, flavonoids, terpenoids, and saponins. The precise site, molecule and cellular mechanisms of the extract remain to be elucidated. The present study supports the traditional use of *C. aromaticus* for its diuretic activity and preferred this drug as compared to other tested drugs. Therefore, it should be used as diuretic in the herbal formulation for diuresis. However, there is a further scope in detail phytochemical investigation and activity guided isolation of active constituents from the plant *C. aromaticus*. The parameters such as phytochemical evaluation and the TLC finger printing profile will help in further qualitative evaluation of *C. aromaticus* in future. In conclusion, our present study is the first scientific study to justify the use of *C. aromaticus* (leaves) as *Pashanbhed* drug as it proved as best

diuretic agent having similar actions to loop diuretics, like furosemide and useful for the identification of *Pashanbhed*, and thus, further support the usage of it in traditional Ayurvedic system of medicine.

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