

# Diuretic activity of leaves of *Plectranthus amboinicus* (Lour) Spreng in male albino rats

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## ABSTRACT

The shade-dried powder of leaves of *Plectranthus amboinicus* (Lour) Spreng was subjected to successive extraction using the various solvents (petroleum ether, chloroform, ethanol and water) in increasing order of polarity. The preliminary phytochemical analyses were carried out for all the extracts. The analyses of the leaves revealed the presence of alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids. Since the phytoconstituents present in the ethanolic and aqueous extracts were similar, both the extracts were selected for further study. The diuretic properties of ethanolic and aqueous extracts were evaluated by determination of urine volume and electrolyte concentration in male albino rats. Furosemide (10 mg/kg) was used as standard while normal saline (0.9%) was used as control. Both ethanolic and aqueous extracts (500 mg/kg) have shown significant increase in the volume of urine and urinary concentration of Na, K and Cl ions. Thus, from the is study it may be concluded that the leaves of *P. amboinicus* (Lour) Spreng possess diuretic activities.

**Key words:** Diuretic, electrolyte concentration, *Plectranthus amboinicus*

## INTRODUCTION

*Plectranthus amboinicus* (Lour) Spreng belongs to family Lamiaceae, and is known as country borage in English.<sup>[1]</sup> It is a large succulent aromatic perennial herb, shrubby below, hispidly villous or tomentose.<sup>[2]</sup> It is found throughout India, Ceylon and Moluccas.<sup>[3]</sup> Earlier, it was claimed that the leaves were being traditionally used as a diuretic.<sup>[2]</sup> Upon literature review it was found that the plant contains butylanisole,  $\beta$ -caryophyllene, quercetin, ursolic acids, triterpenic acids,  $\alpha$ -pinene,  $\beta$ -pinene, thymol, eugenol, carvacrol, 1,8-cineole,  $\beta$ -phellandrene, *p*-cymene, salvigenin, crisimaritin and chrysoeriol.<sup>[4-9]</sup> Many pharmacological properties have been reported including urolithiasis,<sup>[10,11]</sup> antiepileptic,<sup>[12]</sup> antitumor and antimutagenic,<sup>[13]</sup> neuropharmacological,<sup>[14]</sup> radioprotective effect,<sup>[15]</sup> antioxidant,<sup>[16]</sup> antimicrobial,<sup>[17,18]</sup> antibacterial, antifungal properties.<sup>[19,20]</sup> However, there are no reports

on the diuretic activity of the plant. Hence, the present work was focused to verify the earlier claims of the plant.

## MATERIALS AND METHODS

### Plant collection and authentication

The leaves of *P. amboinicus* (Lour) Spreng were collected from the fields of Kanchipuram, Tamil Nadu. It was authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. A voucher specimen of no. PARC/2007/89 has been deposited in the institute.

### Preparation of extracts

The leaves of *P. amboinicus* (Lour) Spreng were dried in shade. Then the shade-dried leaves were powdered to get a coarse powder. The powdered material was successively extracted with petroleum ether, chloroform, ethanol and water by cold maceration in increasing order of their polarities.<sup>[21]</sup> In addition, the fresh powder was defatted with petroleum ether and extracted with 95% ethanol (72 h) and water (24 h) separately. The extracts were filtered with muslin cloth and the solvent was distilled off. Final traces of solvent were removed under vacuum.

### Phytochemical investigation

The petroleum ether (PEPA), chloroform (CEPA),

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ethanolic (EEPA) and aqueous (AEPA) extracts of *P. amboinicus* were subjected to further preliminary, qualitative phytochemical investigation.<sup>[22]</sup> The percentage yields of PEPA, CEPA, EEPA, AEPA were found to be 2.47, 3.69, 12.2 and 18.1, respectively.

### Experimental animals

Healthy, adult wistar albino male rats of weight 150–200 g were obtained from the animal house, Vel's College of Pharmacy, Pallavaram, Chennai. The animals were maintained in well-ventilated room temperature with natural 12 + 12 h day–night cycle in the propylene cages. The animals were fed with balanced pellet and water *ad libitum*. The animals were housed for 1 week prior to the experiments to acclimatize to the laboratory conditions. The experimental protocol of pharmacological and toxicological studies was reviewed and approved by the Institutional Animal Ethics Committee (CPCSEA). Proposal no. CPCSEA/12-12-00/PH-07-14.

### Acute oral toxicity study

Five wistar albino rats of either sex weighing 150–200 g were dosed with extracts in different concentrations and were observed for any symptoms of toxicity for 48 h as per guideline no. 425 (OECD 2001) and LD<sub>50</sub> was estimated to be >5000 mg/kg. Based on the results obtained from this study, the doses of further pharmacological studies were fixed to be 500 mg/kg.<sup>[23]</sup>

### Diuretic activity

The method described by Lipschitz *et al.*<sup>[24]</sup> was employed for the assessment of diuretic activity. The ethanolic and aqueous extracts of *P. amboinicus* (Lour) were suspended in 1% carboxymethyl cellulose (CMC) for oral administration. The solutions of EEPA and AEPA were prepared at a dose of 500 mg/kg b.wt. Furosemide (10 mg/kg b.wt. p.o.) in 1% CMC was used as the standard diuretic agent.

The animals were fasted for 18 h prior to experiment allowing only water during the fasting period. After completion of the fasting period, 5 ml/100 g b.wt. saline (0.9% NaCl) was administered on the day of the experiment. Twenty four healthy rats were selected and divided into four groups consisting of five rats in each. The drug treatment pattern was as follows:

- Group I (control): received normal saline (0.9%) orally at a dose of 10 ml/kg b.wt.
- Group II (standard): received furosemide orally at a dose of 10 mg/kg b.wt.
- Group III (test 1): received ethanolic extract at a dose of 500 mg/kg b.wt.
- Group IV (test 2): received aqueous extract at a dose of 500 mg/kg b.wt.

After treatment, the animals were kept in a metabolic cage. The urine was collected in measuring cylinder and measured at 5 and 24 h after the dose was administered. The bladder was emptied by pulling the base of the tail of each rat. The total difference in the collected urine volume of the respective test group was compared with the standard group. The sodium and potassium concentrations were measured by flame photometry and chloride was estimated titrimetrically.<sup>[25]</sup>

### Statistical analysis

SPSS (version 13.0) statistical program was used to carry out one-way analysis of variance (ANOVA) on the data, followed by Dunnett's *t*-test. Values are expressed as mean ± SEM of six samples. *P* < 0.01 was considered as significant.

## RESULTS AND DISCUSSION

The phytochemical tests revealed that the PEPA extract contained carbohydrates, tannins and terpenoids; CEPA extract had alkaloids; EEPA and AEPA had alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, quinine, tannins, phenolic compounds and terpenoids.

In acute toxicity, LD<sub>50</sub> was estimated to be >5000 mg/kg. Hence, one-tenth of the LD<sub>50</sub>, i.e., 500 mg/kg was selected for the diuretic activity.

There was significant diuresis (*P* < 0.01) on treatment with single dose of the ethanolic extract (1.37 ± 0.06) and aqueous extract (1.18 ± 0.07) of *P. amboinicus* (Lour) at the dose level of 500 mg/kg b.wt. It was comparable to significant (*P* < 0.01) diuresis produced by standard furosemide (1.41 ± 0.08), when compared with the control rats (0.63 ± 0.10) at 5 h after the dose. Urine output continued to be stimulated throughout the study period such that the cumulative urinary excretion was found significant (*P* < 0.01) in rats treated with furosemide (2.22 ± 0.05), EEPA (2.15 ± 0.07) and AEPA (2.02 ± 0.11) when compared with the control (1.05 ± 0.08) after 24 h of drug administration. The results are presented in Table 1.

The effects of single dose of furosemide 10 mg/kg b.wt. and 500 mg/kg b.wt. of the EEPA and AEPA of *P. amboinicus* Lour. on electrolyte (Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup>) excretion in the 24-h urine were estimated and are presented in table 1. Furosemide, EEPA and AEPA significantly (*P* < 0.01) enhanced the excretion of the Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> as compared to the control group. The results clearly indicate that the diuretic action produced by EEPA was quite closer to the standard diuretic furosemide.

The diuretic activity of *P. amboinicus* (Lour) may be due to

**Table 1: Diuretic activity of ethanolic and aqueous extracts of *P. amboinicus* (Lour) Spreng**

Treatment	Dose	Volume of urine (ml/100 g)		Urinary electrolyte concentration (24 h)		
		5 h	24 h	Na <sup>+</sup> (mEq/l)	K <sup>+</sup> (mEq/l)	Cl <sup>-</sup> (mEq/l)
Control	10 ml/kg	0.63 ± 0.10	1.05 ± 0.08	86.83 ± 0.67	44.18 ± 0.89	53.97 ± 0.98
Furosemide	10 mg/kg	1.41 ± 0.08*	2.22 ± 0.05*	138.17 ± 0.79*	71.89 ± 2.27*	98.92 ± 1.24*
Ethanolic extract	500 mg/kg	1.37 ± 0.06*	2.15 ± 0.07*	135.14 ± 1.13*	69.68 ± 1.19*	91.28 ± 2.62*
Aqueous extract	500 mg/kg	1.18 ± 0.07*	2.02 ± 0.11*	121.73 ± 1.46*	60.13 ± 1.56*	81.54 ± 0.95*

Values are expressed as mean ± SEM of six samples. Data were analyzed by one-way ANOVA followed by Dunnett's *t*-test.

\*represents *P* < 0.01 when compared to control.

the individual or combined action of bioactive constituents present in it. Further phytochemical and pharmacodynamic investigations are required to find the active constituents responsible for the activity and to understand the precise mechanism of diuretic exhibited by aqueous and ethanolic extract of leaves of *P. amboinicus* (Lour) Spreng.

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