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Megavarthini R
PG Scholar, Department of Pharmacognosy - The Erode College of Pharmacy, Erode, Tamil Nadu, India. Affiliated to the Tamil Nadu Dr. M.G.R University, Guindy, Chennai, Tamil Nadu, India

Mahalaksmi S
PG Scholar, Department of Pharmacognosy - The Erode College of Pharmacy, Erode, Tamil Nadu, India. Affiliated to the Tamil Nadu Dr. M.G.R University, Guindy, Chennai, Tamil Nadu, India

Gokulakrishnan S
PG Scholar, Department of Pharmacognosy - The Erode College of Pharmacy, Erode, Tamil Nadu, India. Affiliated to the Tamil Nadu Dr. M.G.R University, Guindy, Chennai, Tamil Nadu, India

Manokaran S
Professor and Head, Department of Pharmacognosy - Grace College of Pharmacy, Palakkad, Kerala, India

Ravikkumar VR
Professor & Head, Department of Pharmacognosy, the Erode College of Pharmacy, Erode, Tamil Nadu, India. Affiliated to the Tamil Nadu Dr. M.G.R University, Guindy, Chennai-12, Tamil Nadu, India

Corresponding Author:
Megavarthini R
PG Scholar, Department of Pharmacognosy - The Erode College of Pharmacy, Erode, Tamil Nadu, India. Affiliated to the Tamil Nadu Dr. M.G.R University, Guindy, Chennai, Tamil Nadu, India

Anti-urolithiatic activity of hydro-alcoholic extract of the whole plant *Pennisetum purpureum* (*Cenchrus purpureus*)

Megavarthini R, Mahalaksmi S, Gokulakrishnan S, Manokaran S and Ravikkumar VR

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Abstract
The goal of this study was to evaluate the anti-urolithiatic potential of the hydro-alcoholic extract of the whole plant of *Pennisetum purpureum* (HAEPP). Soxhlet extraction using water and ethanol (1:1) yielded 14.8% w/w of extract, which was found to contain carbohydrates, tannins, saponins, flavonoids, alkaloids, and triterpenoids. *In vitro* pharmacological screening was conducted using calcium oxalate crystals as a model for kidney stone formation. Nucleation, aggregation, and titrimetric dissolution assays were performed to assess efficacy. HAEPP at 200 mg/ml significantly inhibited nucleation (76%) and crystal aggregation (60%), and effectively dissolved calcium oxalate stones (82.90%), results comparable to the standard drug Cystone. These activities are likely attributed to the presence of saponins, tannins, and triterpenoids, which interfere with crystal formation, aggregation, and adhesion. Overall, the study demonstrates that HAEPP exhibits potent *in vitro* anti-urolithiatic activity. Its availability, low cost, and significant efficacy highlight its potential as a natural therapeutic agent for the prevention and management of calcium oxalate kidney stones.

Keywords: *Pennisetum purpureum*, HAEPP, Anti-urolithiatic, Nucleation & Aggregation assay, titrimetric method

Introduction

"Herbal medicine is a true gift from nature, providing safe and effective treatments for human health. Plants have long been used to treat illness and promote well-being. Even today, herbal remedies are essential for preserving overall health and illness prevention.

Urolithiasis is the production or deposition of calculi in the urinary tract, and it is the third most frequent condition, affecting around 12% of the global population [1, 2]. The formation of calcified renal stones is a physiochemical event that involves crystal nucleation, aggregation, and growth, which is aided by a variety of biological processes such as urine volume, pH, increased calcium or sodium oxalate, and urates [3, 4]. Currently, more than 90% of upper urinary tract stone patients have been treated based on the size, kind, and position of the stones, with a success rate of 68 to 86% [5].

Increased dietary protein intake has been linked to an increased risk of kidney stone formation, with calcium oxalate and/or calcium phosphate accounting for roughly 75% of all renal stones [6]. Although technological progress has been impressive in the treatment/management of kidney stones, the related complexity, such as their potential to promote recurrence, haemorrhage, hypertension, and tubular nephrosis, remain a major limiting factor [7]. As a result, alternative or complementary medicines with little side effects could be beneficial. Herbal therapy is gaining popularity for the treatment of urolithiasis in both developed and developing countries due to its broad biological and therapeutic properties, low toxicity, and low cost [1, 8].

Pennisetum purpureum, also known as "elephant grass," is native to African countries including Kenya, Ethiopia, Cameroon, Cote D'Ivoire, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, Angola, Malawi, Mozambique, Tanzania, Togo, Uganda, Zambia, and Zimbabwe.

Pennisetum purpureum is used ethno-pharmacologically as a laxative in Gambia, a tranquillizer in Tanganyika, a diuretic and painkiller in Nigeria, and a mouthwash for buccal infections, gingivitis, and thrush in Congo [9]. *P. purpureum* is a hardy perennial with a deep root system that can reach 4.5 meters. The plant belongs to the *Poaceae* family, has been extensively utilised in cattle nutrition, and has enormous potential as a feedstock for biofuel manufacturing. In Kenya, over 70% of livestock farmers cultivate *Pennisetum purpureum* [10].

Plant Profile

Fig 1: *Pennisetum Purpureum*

Plant: *Cenchrus Purpureus*

Synonyms: *Pennisetum purpureum*

Common name: Napier grass elephant grass or Uganda grass [11].

Family: *Poaceae*

Description

Robust perennial grass that grows in thick clumps or colonies from basal offshoots or short rhizomes, reaching heights of up to 4 metres. Stems are frequently branched above; internodes are more or less bluish glaucous; young nodes have white hairs, which later become smooth and glabrous. The leaf sheaths are glabrous and usually shorter than the internodes, while the ligule has a narrow rim densely fringed with long white hairs. Leaf blades linear to tapering, flat, typically bluish green, up to 1 m long and 3 cm wide, pilose towards the base, particularly on margins; blade margins often rough; midvein stout, whitish above, prominently keeled below.

The inflorescence is a thick terminal bristly spike that is tawny to purple-tinged and measures about 20 cm long and 2 cm broad. Spikelets are 4-6 mm long, solitary or in clusters of 2-6 on a hairy axis, encircled by sparsely plumose bristles to 2 cm long that fall with the spikelets when mature; the outermost glume is minute or missing [12].

Chemical constituents

The phytochemical examination of *P. purpureum* revealed the presence of tannins (67.9%), alkaloids (lunamarine 26.21%), phenols (1.16%), and flavonoids (rutin 2.10%, anthocyanin 0.06%, catechin 2.49%, and kampferol 0.09%) [13].

Uses

Pennisetum purpureum, sometimes known as Napier grass, has a long history of medicinal and ethno-veterinary uses. In African folk medicine, it is commonly used as a diuretic to increase urine flow and treat urinary disorders such as kidney stones [14, 15]. Decoctions of its leaves and roots are used to treat fevers, inflammation, and pain [14, 16]. The herb is also used externally to treat wounds, burns, and gingivitis, demonstrating its antibacterial and anti-inflammatory qualities [16]. In some traditional cultures, it is used as a laxative to promote digestion and treat constipation, as well as a sedative and stress reliever [17].

Materials and Methods

Plant Collection and Drying

The plant *Cenchrus purpureus*, synonym *pennisetum purpureum*, known in Tamil as Elephant Grass or Yannai Pull, was collected in Veruvadampalayam village, Tirrupur District, Tamil Nadu. The botanical survey of India, Coimbatore, authenticated the plant as BSI/SRC/5/23/2022/Tech/634,635,636. The plant parts of *Cencherus purpureus*, such as the leaves and stem, were harvested and shade dried for 7 to 15 days. After drying, dried plant components were blended to produce homogenous powdered particles suitable for extraction.

Extraction

Approximately 100g of dried plant material was roughly pulverised and placed in a thimble within a Soxhlet extractor. The extraction solvent was a 1:1 mixture of ethanol and water, which was put to a round-bottom flask connected to the extractor and condenser. The setup was heated and left to run continuously for 48 hours until the solvent in the syphon tube turned colourless, indicating that extraction was complete. The resulting extract was then concentrated with a rotary evaporator and dried to produce the crude extract. The concentrated extracts were then weighed to determine the yields, and the percentage yield for each extract was calculated.

Fig 2: Soxhlet Extraction of *Pennisetum Purpureum*

Phytochemical Screening

Standard phytochemical procedures were used to examine each of the *P. purpureum* extracts. Each concentrated

extract was subjected to qualitative tests using conventional protocols [18, 19] to determine the presence of alkaloids, carbohydrates, flavonoids, saponins, tannins, and terpenoids.

Anti-Urolithiatic Activity [20-33]

Nucleation Assay (Turbidity Method)

The *in vitro* anti-urolithiatic activity of the plant extract was evaluated using the modified method of Prachi Khare *et al*. Calcium oxalate (CaOx) crystallization was induced by mixing calcium chloride and sodium oxalate in Tris buffer (pH 6.5) at 37°C, and turbidity was measured at 620 nm using a UV–Vis spectrophotometer. For the control, 0.95 ml of 50 mM $CaCl_2$ was mixed with 1 ml distilled water, followed by 0.95 ml of 75 mM sodium oxalate; after shaking for 1 min, absorbance was recorded at 1-min intervals for 5 min. The test samples were prepared by replacing distilled water with plant extract solutions at concentrations of 100, 250, and 500 µg/ml, while the standard (Cystone) was tested at the same concentrations. Percentage inhibition of CaOx nucleation was determined from the slopes of absorbance values using the formula:

$$\% \text{ Inhibition} = (1 - S_i/Sc) \times 100,$$

where S_i is the slope in the presence of inhibitor (extract or standard) and Sc is the slope of the control.

Aggregation Assay

Semi-permeable membranes were prepared from hen eggs by piercing the apex with a glass rod, flushing out the contents with distilled water, and immersing the cleaned shells overnight in 4 ml concentrated HCl diluted in 200 ml distilled water for decalcification. The following day, membranes were carefully removed, washed thoroughly, neutralised with ammonia solution, rinsed with distilled water, and incubated at 37°C for 30 min. For the standard, a 500 mg Cystone tablet was de-coated with ethanol, yielding 400 mg of powder, which was dissolved in 100 ml distilled water and used as a positive control, while 10 mg of calcium oxalate in 10 ml purified water served as the negative control. Synthetic calcium oxalate was prepared by dissolving 1.47 g calcium chloride dihydrate in 100 ml distilled water and 1.34 g sodium oxalate in 100 ml of 2N H_2SO_4, followed by mixing under constant stirring. The precipitate was washed with ammonia solution, rinsed with distilled water, and dried at 60°C for 2 h.

For the assay, experimental groups were set as:

- **Group I:** Control
- **Group II:** 1ml of Calcium Oxalate (1mg/ml) + 1ml of Cystone Solution
- **Group III:** 1ml of Calcium Oxalate (1mg/ml) + 1ml Extract (100mg/ml)
- **Group IV:** 1 ml of Calcium Oxalate (1mg/ ml) + 1ml Extract (200mg/ml)
- **Group V:** 1 ml of Calcium Oxalate (1mg/ ml) + 1ml Extract (300mg/ml)
- **Group VI:** 1 ml of Calcium Oxalate (1mg/ ml) + 1ml Extract (400mg/ml)

Semi-permeable membranes were filled with the respective test solutions, knotted with thread, and suspended in conical flasks containing 150 ml of 0.1 M Tris buffer. The flasks were covered with aluminium foil and incubated at 37°C for 3 days. After incubation, the contents were collected, treated with 4 ml of 1N H_2SO_4 and 60–80 ml of 0.02 M $KMnO_4$, and allowed to stand for 2 h until the solution decolourised from dark pink to colourless. Absorbance was recorded at 620 nm using a UV–Vis spectrophotometer, and the concentration of undissolved calcium oxalate was calculated from a standard calibration curve.

Calcium oxalate measured by Titrimetry

To determine the dissolution percentage of calcium oxalate, 1 mg of calcium oxalate and 10, 20, 30, or 40 mg of extract were packed in a semi-permeable egg membrane. This was allowed to suspend in a conical flask with 100 cc of 0.1M Tris buffer. The first group served as a blank, containing merely 1 mg of calcium oxalate. The second group served as a positive control, containing 1 mg of calcium oxalate in addition to 600mcg, 800mcg, and 1mg of the standard drug, cystone. The third and fourth groups were combined with 10 mg of calcium oxalate-containing hydro-alcoholic extracts.

All groups' conical flasks were stored in an incubator set to 37°C for 2 hours. Remove the contents of each group's semipermeable membranes into separate test tubes, add 2 ml of 1N sulphuric acid to each, and titrate with 0.9494 N KMnO4 until a light pink hue is obtained. The remaining undissolved calcium oxalate is subtracted from the total quantity utilised in the experiment at the start to determine the total amount of dissolved calcium oxalate by various solvent extracts.

Each ml of 0.9494 N KMnO4 is equivalent to 0.1898 mg of calcium oxalate.

Result

Extraction yield of *pennisetum purpureum*

Extraction was carried out using water and ethyl alcohols (1:1) as solvents in the Soxhlet extraction technique for 48 hours, after which the solvent was evaporated and the extract was stored in a desiccator for future use. The extraction yielded 14.8% w/w.

Phyto-chemical Evaluation

The phytochemical examinations of the aforesaid extract revealed the presence of carbohydrates, tannins, saponins, flavonoids, alkaloids, and triterpenoids. Table 1 shows a list of phyto-constituents found in the hydro-alcoholic extract of the complete plant of *Pennisetum purpureum.*

Table 1: List of phyto-constituents present in the hydro-alcoholic extract of the whole plant of *Pennisetum Purpureum*.

S. No	Phytoconsistuents	Remark
1	Tannins	+
2	Saponins	+
3	Flavonoids	+
4	Carbohydrates	+
5	Alkaloids	+
6	Triterpenoids	+

***In-Vitro* Pharmacological Screening**

Nucleation Assay

In the nucleation assay, the percentage inhibition of different extract concentrations (100mg/ml, 200mg/ml, 300mg/ml, and 400mg/ml) was compared to the usual cystone dosage of 400 mg/ml. Figure 3 depicts the results.

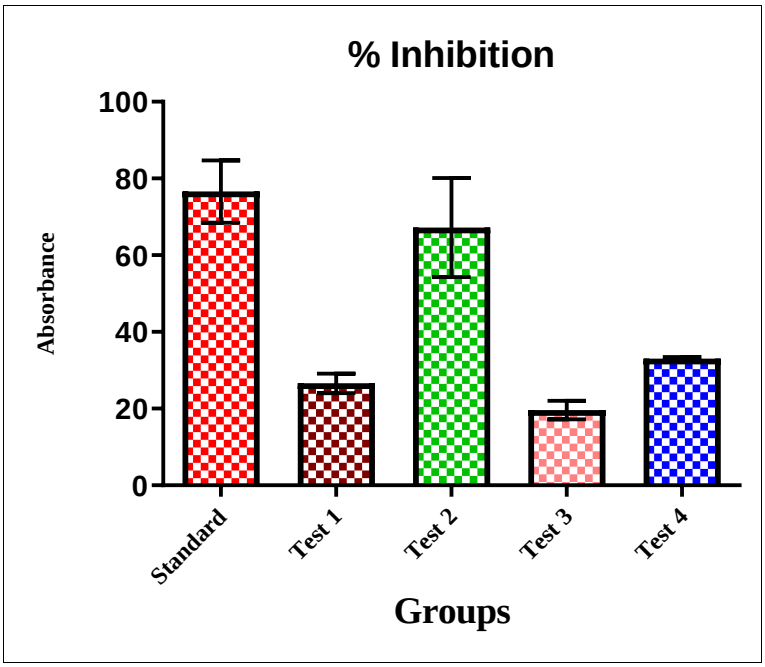

Fig 3: % Inhibition of different concentration of extract compared with standard cystone.

Aggregation Assay

In the aggregation assay, the percentage inhibition of various extract concentrations (100mg/ml, 200mg/ml, 300mg/ml, and 400mg/ml) were compared to the standard cystone concentration of 400mg/ml. Figure 4 depicts the results.

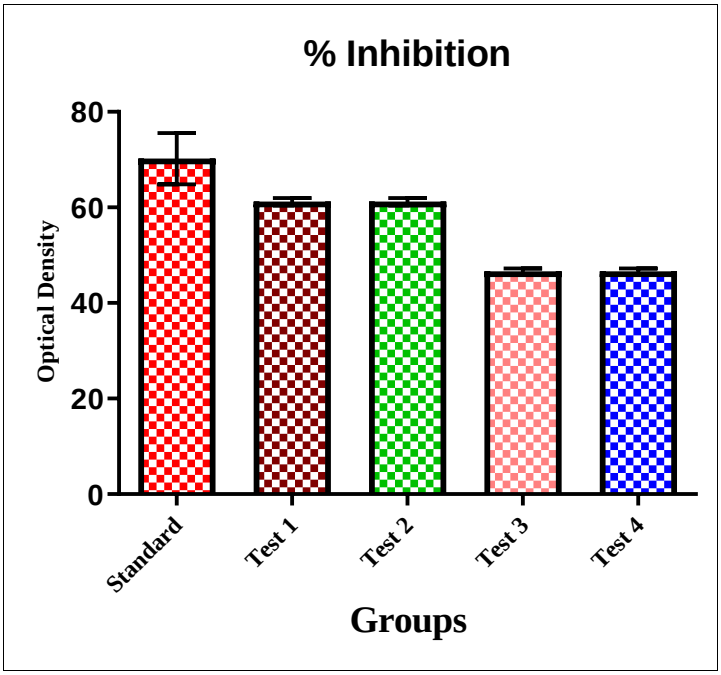

Fig 4: % Inhibition of different concentration of extract compared with standard cystone.

Titrimetric Method

The percentage of dissolution of calcium oxalate stone by titrimetric method was performed by using $KMnO_4$ was presented in the table 2.

Table 2: % Dissolution of calcium oxalate by titrimetric method

S. No	Compounds	% of inhibition
1	Cystone + Calcium Oxalate	83.02%
2	100 mg of extract + 10 mg of calcium oxalate	82.9%
3	200 mg of extract + 10 mg of calcium oxalate	81.02%
4	300 mg of extract + 10 mg of calcium oxalate	79.12%
5	400 mg of extract + 10 mg of calcium oxalate	75.32%

The results show that the hydro-alcoholic extract of *pennisetum purpureum* (100mg/ml & 200mg/ml) significantly (82.90% & 81.02%) dissolved the formed calcium oxalate stones when compared to the standard cystone (83.22%).

Discussion

The hydro-alcoholic extract of *Pennisetum purpureum* (HAEPP) was obtained with a yield of 14.8% w/w and was found to contain carbohydrates, tannins, saponins, flavonoids, alkaloids, and triterpenoids. In the nucleation assay, HAEPP exhibited 76% inhibition at 300 mg/ml, which was comparable to the standard Cystone (78%), suggesting that the presence of saponins may contribute to the disruption of mucoprotein-mediated calcium oxalate crystallization. In the aggregation assay, HAEPP at 100 and 200 mg/ml inhibited crystal aggregation by 60%, which was close to the standard (69%), indicating its ability to prevent the conversion of calcium oxalate monohydrate (COM) into calcium oxalate dihydrate (COD), thereby reducing the risk of stone adherence to renal epithelium. The titrimetric assay further revealed that HAEPP (100 and 200 mg/ml) dissolved calcium oxalate stones by 82.90% and 81.02%, respectively, comparable to Cystone (83.02%), which may be attributed to the activity of triterpenoids and tannins. Overall, HAEPP at 200 mg/ml demonstrated the most significant antiurolithiatic potential across nucleation, aggregation, and dissolution assays, highlighting its therapeutic relevance in the management of calcium oxalate urolithiasis.

Conclusion

Cenchrus purpureus, also known as *Pennisetum purpureum*, is a well-known cattle feed that grows well in all parts of India and is the least expensive economically. The hydro-alcoholic extract of the entire plant was examined for its physicochemical and phytochemical ingredients, and it was confirmed to be safe with a number of phytochemicals. HAEPP is a strong inhibitor of calcium oxalate stone nucleation as well as stone aggregation in an *in vitro* model. The extract's dissolving characteristics were noteworthy in terms of stone dissolution. Further research would be required to demonstrate the plant's anti-urolithiatic effectiveness *in vivo* in order to transform the abundantly accessible inexpensive plant into a cost-effective medicine for the impoverished people to treat kidney stones.

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