

Assessment of Anti-Lithiatic Activity in *Pedaliium murex* L as A Binding Agent in Calcium Oxalate and Magnesium Trisilicate Tablets

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Cite this paper as: Geetha. G, Ranjithkumar. M, Sarathi. A, Thendral raj. P, (2025) Assessment of Anti-Lithiatic Activity in *Pedaliium murex* L as A Binding Agent in Calcium Oxalate and Magnesium Trisilicate Tablets. *Journal of Neonatal Surgery*, 14 (32s), 8618-8626.

ABSTRACT

One of the oldest diseases that humans have ever encountered is renal lithiasis, which was first recorded in ancient Greek. Renal lithiasis is the result of a change in the typical crystallization conditions of urine in the urinary system. Kidney stones are frequently caused by calcium, phosphate, and oxalate. Developing an oral herbal dose formulation of *Pedaliium murex* L as a binding agent in calcium oxalate and magnesium trisilicate tablets with assessed anti-urolithiatic activity was the main goal of this investigation. Phytochemical screenings of *Pedaliium murex* L leaf extracts showed presence of flavonoids, Alkaloids, glycosides, steroids, phenol, saponins, and tannins. *Pedaliium murex* L Aerosil, microcrystalline cellulose, cross povidone as a disintegrating agent, sodium starch glycolate, cross-linked sodium carmellose as a filler, and powdered *Pedaliium murex* L mucilage as a binder were used to create the solid oral herbal dosage form, tablets. The angle of repose was found between 28.32 and 29.71, the range of bulk density was 0.47–0.49 mg/ml, and Density Tapped was 0.51–0.57 mg/ml was calculated. Hausner's ratio ranged from 1.15 to 1.29, and Carr's index was computed. The formulations' powder mixes showed good flow ability with Hausner's ratios of 1.2 or less. The powder blend's showed good flow ability was further demonstrated by the angle of repose, which is below 30 and ranges from 24 to 29. The research findings demonstrated the herbal tablet formulation potential preventing qualities against calcium oxalate crystal.

Keywords: *Pedaliaceae, Bada Gokhru, Galbanum, Wet Granulation, Resin, phytochemical screening.*

1. INTRODUCTION

One of the oldest diseases that humans have ever encountered is renal lithiasis, which was first recorded in ancient Greek. The remains of 7000-year-old Egyptian mummies have been discovered to contain urinary stones. According to Mute et al. (2008), renal lithiasis is the result of a change in the typical crystallization conditions of urine in the urinary system. The lifetime chance of developing stones is more than 6-9% for males and 3-4% for women, indicating the prevalence of stone disease. [1]According to Adriano et al. (2000), the general likelihood of producing stones varies by region of the world, ranging from 1 to 5% in Asia, 5 to 9% in Europe, 13% in North America, and 20% in Saudi Arabia. [2] Kidney stones are frequently caused by calcium, phosphate, and oxalate. Urine volume, the concentration of stone ingredients (a function of urine volume), the existence of a nidus, and the equilibrium between different physicochemical parameters that either prevent or promote stone formation are the factors that affect crystal creation. The kidneys are crucial for conserving water, but they also eliminate minerals that are poorly soluble. Urinary stones or kidney stone are created when the usual balance of water, salt, minerals and other components found in the urine is altered. [3] Kidney stones can result from a variety of conditions, including gout, hypercalciuria, hyperparathyroidism, renal tubular acidosis, and some inherited metabolic disorders like cystinuria and hyperaluria. [4], [28] Kidney stones can result from some medications, such as diuretics, calcium-containing antacids, ephedrine, guaifenesin, and protease inhibitors. Indinavir is a medication used to treat HIV infection. [5], [29].

The majority of kidney stones are caused by calcium combined with oxalate, phosphate, and uric acid. [6]Since oxalate is a naturally occurring substance, it can be found in a variety of foods, including chocolate, nuts, fruits, and vegetables. The liver also produces it metabolically. [7]A healthy adult's normal oxalate level is between 20 and 40 mg/d. Increased levels of oxalate and calcium in the urine are caused by a variety of dietary factors, including high vitamin D intake, an oxalate-rich diet, metabolic diseases, and intestinal bypass surgery. Calcium phosphate and calcium oxalate stones are white, grey,

or black in color, giving them a radio-opaque appearance.[8] They are around 1 cm in diameter and show up as dense, strongly outlined structures on radiographs. Calcium phosphate stones have been linked to medical problems such as hyperparathyroidism and renal tubular acidosis. [9] About 5–10% of kidney stones are yellow-orange, spherical, smooth stones composed of uric acid; these stones show almost clear on radiographs unless they have been combined with struvite or calcium crystals. [10] These stones are commonly square, diamond or rod shaped, pleomorphic crystals which are polarizable. Uric acid kidney stones are found in people with disorders such as gout syndrome, obesity, or those who consume a diet high in proteins and purines, particularly meat and fish. [11]

Triple phosphate, also known as struvite, stones are a crystalline material composed of magnesium ammonium phosphate that make up approximately 10-15% of kidney stones. They are generated when a bacterial infection is present. The bacterial enzyme urease, which breaks down bacteria into ammonia and carbon dioxide and makes urine alkaline, promotes the formation of struvite stones. Large, glaring, and layered struvite stones are typically encountered in people with specific metabolic disorders, such as gout, idiopathic hypercalciuria, and hyperparathyroidism. [12] Rare genetic conditions called cystinuria, which cause the kidneys to release too many amino acids, are the cause of these stones. These greenish-yellow stones have a spherical, moderately radio-opaque look and are speckled with gleaming crystallites. [13]

Anti-lithiasis refers to the prevention and treatment of kidney stones, which are hard mineral and salt deposits that form in the kidneys and can cause severe pain and complications. These stones can vary in size and composition and may block the urinary tract, leading to discomfort, infections, and impaired kidney function. Anti-lithiasis strategies typically include dietary modifications, increased fluid intake, and medications designed to dissolve or prevent the formation of these stones. Intake of higher amount of calcium in food results in Lithiasis occurs due to the settle down of calcium salts in Urinary Tract. The Magnesium trisilicate and calcium oxalate tablet causes lithiasis as a side effect. [14]



Fig 1:

Pedaliium Murex L commonly known as Bada gokhru belongs to family pedaliaceae is one of the most useful traditional medicinal plants in India. The leaves contain the gum resin “galbanum”. The galbanum gum reported for its anti-lithiatic activity. This study gave gum resin was used as a binding agent in magnesium trisilicate and calcium oxalate tablet formulation such as to overcome the lithiatic effect. [15]

With chromosomal number $n=8$, *Pedaliium murex L*. is a succulent, glandular, annual creeper that grows 2–3 feet long and has branches all over. In pairs of five to eight, the leaves are simple, opposite, ovate, glabrous, alternating, fleshy, estipulate, petiolate, and irregularly shaped. Their length and width range from 4.0 to 6.5 cm and 4.0 to 5.0 cm, respectively. [16]



Fig: 2

2. MATERIAL AND METHODES

Extraction of mucilage

Pedaliu murex L fresh leaf (100g) was soaked in distilled water (200 ml). Heat it with stirring at 50 C for 2 hrs. The dissolved solution was separated using a muslin cloth. [17] Solution was centrifuged at 4000 rpm for 2 hrs. The supernatant was washed in (ratio 5:2) using ethyl alcohol and acetone for removing chlorophyll by centrifuging (2700 rpm, 15min).subsequently, the precipitated mucilage was dried in an oven at 40 C for 2 hrs and crushed using a blender. The powdered *pedaliu murex l* mucilage was sealed in a container and stored. [18].

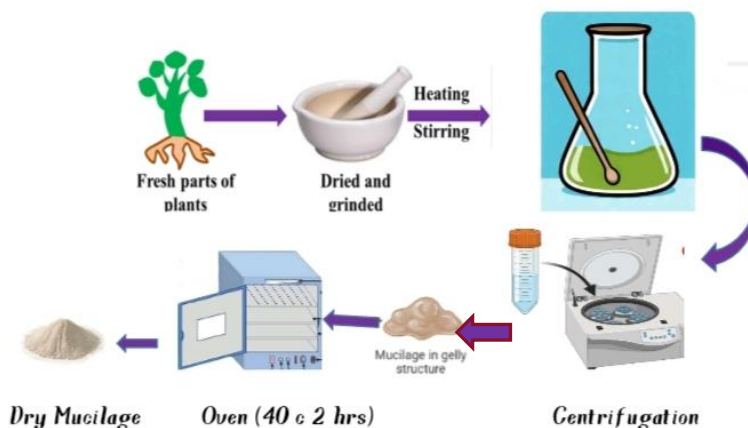


Fig: 3



PHYTOCHEMICAL SCREENING: Table: 1

Using established techniques, the content of flavonoids, alkaloids, glucosides, steroids, phenol, saponins, terpenoids, cardiac glycosides, and tannins was examined in *Pedaliu Murex* leaves extracts. [19], [20], [21], [50], [52], [54].

Steroids (Salkowski's test):

A dry extract of *Pedaliu murex* weighing about 100 mg was dissolved in 2 milliliters of chloroform. Sulfuric acid was carefully added to generate a lower layer. A reddish brown hue at the interface suggested the presence of a steroidal ring.

Cardiac glycosides (Keller killiani's test):

About 100 mg of extract were dissolved in 1 milliliter of glacial acetic acid with a drop of ferric chloride solution. Below this, 1 milliliter of sulfuric acid concentrate was applied. A brown ring that was produced at the contact indicated the presence of the deoxy sugar that defines cardenolides.

Saponins:

A drop of sodium bicarbonate was put into a test tube containing around 50 milliliters of an aqueous sample extract. Three combinations were left after the mixture had been thoroughly shaken. A froth that looked like saponins were present.

Resins:

Two milliliters of ethanolic extract or chloroform were mixed with five to ten milliliters of acetic anhydride, and the mixture was then gently heated to dissolve it. After cooling, 0.5 cc of H₂SO₄ was added. The outcome was a striking shade of purple. It demonstrated the presence of resins.

Phenols (Ferric Chloride Test):

After mixing 1 milliliter of the sample's alcoholic solution with 2 milliliters of distilled water, a few drops of a 10% aqueous ferric chloride solution were added. When a blue or green color developed, phenols were detected.

Tannins (Lead acetate test):

A test tube containing around 5 ml of an aqueous extract was treated with a few drops of a 1% lead acetate solution. The development of a reddish-yellow precipitate indicated the existence of tanning.

FeCl₃ test:

Two milliliters of the filtrate 200 milligrams of plant material in ten milliliters of distilled water and two milliliters of FeCl₃ were combined. A blue or black precipitate indicated the presence of tannins.

Terpenoid:

A reddish brown color was used to identify the presence of terpenoid when 1 mg of extract was added to 2 ml of chloroform and 1 ml of saturated H₂SO₄.

Glycosides:

A small amount of the sample's alcoholic extract was dissolved in 1 milliliter of water, and then aqueous sodium hydroxide was added. The yellow color formulation indicated the presence of glycosides.

Flavonoids:

Five to ten drops of diluted HCl were added, along with a tiny bit of magnesium or zinc. A test tube containing 5 milliliters of the sample's alcoholic extract was heated for a few minutes. Flavonoids were identified by their dirty brown or reddish-pink color. [55]

Alkaloids (Mayer's test):

1.36 grams of mercuric chloride and 5 grams of KI were dissolved in 60 milliliters of distilled water, respectively. These two solvents were mixed and diluted to 100 milliliters using distilled water. One milliliter of an acidic aqueous solution of the materials was mixed with a few drops of the reagent. A whitish or pale precipitate indicated the presence of alkaloids.

Confirmation test for mucilage:

Ruthenium red test:

A tiny amount of dry mucilage that contains it is put in a slide. They add a ruthenium red solution. A microscope is used to view the sample. Mucilage will stain pink or purple if it is present. [22]



Fig: 5

Preparation of mixed blend of drug and excipients. Table: 2

Aerosil, microcrystalline cellulose, cross povidone, sodium starch glycolate, cross-linked sodium carmellose, and powdered *pedalium murex* L mucilage were all run through mesh number 60. For every given formulation, the necessary amount of each ingredient was taken, and each ingredient was ground to the necessary level of fineness. The following flow characteristics of the powder blend were assessed. [23]

Angle of Repose

We used the funnel method to determine the angle of repose. The mixture was poured through a vertically adjustable funnel until the maximum cone height (h) was reached. The formula was used to determine the angle of repose (q) and measure the heap's radius ®.

$$\bullet = \tan^{-1} (h/r)$$

Bulk Density

The blend was poured into a graduated cylinder to obtain the apparent bulk density (b). The powder's weight (M) and bulk volume (V) were calculated. The formula Bulk Density ($\bullet b$) = M/V was used to determine the bulk density.

Tapped Density

For a predetermined amount of time, the measuring cylinder holding a known mass of mix was tapped. The blend's weight (M) and minimum volume (VT) in the cylinder were calculated. The following formula was used to determine the tapped density ($\bullet t$): $M/VT = \text{Tapped Density } (\bullet t)$.

Hausner Ratio

An indirect measure of powder flow easiness is the Hausner ratio. This formula is used to calculate it: $t/d = \text{Hausner ratio}$. Better flow characteristics are indicated by a lower Hausner ratio (<1.25) than by a higher one (>1.25), where **t** is the tapped density and **d** is the bulk density.

FORMULATION OF TABLETS:

With the exception of aerosil, all of the materials listed in were combined thoroughly to achieve the necessary level of fineness. Lastly, aerosol was stirred in. [23] a single-punch tablet punching machine was used to compress the mixture of medication and excipients to create 150 mg tablets with an 8 mm diameter. [24].



Fig: 6

EVALUATION OF TABLETS: Table: 3

The three tablet forms (T1, T2, and T3) were assessed for appearance, friability, hardness, weight fluctuation, and disintegration.

In-vitro Anti-lithiatic activity of tablet formulation. Table: 4

Nucleation Assay

The extracts were evaluated for their ability to suppress CaOx crystal nucleation using a spectrophotometric assay. [25] The crystallization process was initiated by adding calcium chloride (4 mol/L) and sodium oxalate (50 mol/L) solutions to artificial urine that had been prepared in a buffer with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 and 37 °C. [51] The rate of nucleation was determined by comparing the induction time of crystals the amount of time it took for the crystals to reach a critical size and become optically detectable with that of the control, which contained no extract. [26] The absorbance (optical density, or OD) obtained at 620 nm was used to calculate the percentage inhibition, which was expressed as $(OD(\text{experimental})/OD(\text{control}))/100$. [27]

3. RESULT AND DISCUSSION

Phytochemical screenings of *Petalium murex l* leaf extracts

Phytochemical constituents		Petroleum ether	Chloroform	Acetone	ethanol
Flavonoids		-	-	-	+
Alkaloids		-	-	-	+

Glycosides		-	+	+	+
Steroids		-	-	-	+
Phenols		-	-	+	+
Terpenoid		-	-	-	+
Saponins		-	+	+	+
Resins		-	-	-	-
Tannins	FeCl ₃ test	-	-	+	+
	Lead acetate test	-	-	-	+
Cardiac glycosides		-	-	-	-

Phytochemical screenings of *Pedaliu murex* leaf extracts: Table: 1

(- Absent) (+ Present)

Evaluation of Powder Blend.

Batch	Angle of repose	Carr's index (%)	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner's ratio
T1 (mg)	28.32	17.22	0.47	0.51	1.23
T2(mg)	30.56	15.67	0.43	0.58	1.15
T3(mg)	29.71	16.93	0.49	0.57	1.19

Evaluation of powder blend: Table: 2

EVALUATION OF PREPARED TABLET.

Batch	Hardness (Kg/cm ²)	Thickness (mm ²)	(%)weight Variation	(%) Friability W/W	Disintegration (sec)
T1 (mg)	10	3.00	2.11	0.1	15
T2 (mg)	6	3.20	2.16	0.2	10
T3 (mg)	8	3.17	2.21	0.2	12

Evaluation of Prepared tablet: Table: 3

In-vitro Anti-lithiatic activity of tablet formulation.

Sample	Concentration (ug)	absorbance@620 nm	Percentage inhibition
Cystone (standard drug)	100ug	0.38	62%
Pedaliu murex L	100ug	0.54	46%

Nucleation Assay: Table: 4

4. RESULTS

The primary objective of this work was to develop oral herbal dosage formulation of *Pedaliu murex L* as a binding agent in calcium oxalate and magnesium trisilicate tablets with evaluated of anti-urolithiatic activit. The development of such formulations will mark an important advancement in the area of phytopharmaceuticals. The present investigation examines

design & development of solid oral herbal dosage form were prepared by using

Pedaliu murex L. The solid oral herbal dosage form, tablets were prepared using Aerosil, microcrystalline cellulose, cross povidone as a disintegrating agent, sodium starch glycolate, cross-linked sodium carmellose as filler, and powdered ***Pedaliu murex L*** mucilage as a binder. Blend of drug and excipients were prepared and evaluated by various parameter. Angle of Repose was found in range 28.32-29.71 for 3 tablet. Bulk Density was found in range 0.47-0.49 mg/ml .Tapped Density 0.51-0.57 mg/ml .Hausner's ratio was found range between 1.15 -1.29 for 3 tablet and carr's index was calculated. The powder blends of all the formulations had Hausner's ratio of 1.2 or less indicating good flow ability (Tang et al., 2001). The good flow ability of the powder blend was also evidenced with angle of repose (range of 24-29) which is below 30 indicating good flow ability. The tablet was prepared by using single-punch tablet punching machine was used to compress the mixture of medication and excipients to create 150 mg tablets with an 8 mm diameter. All the parameters were found well within the specified limit for uncoated tablets. T1, T2 and T3 form of tablets was good quality with regard to angle of repose and bulk density and Hauser's ratio.

The *in-vitro* antilithiatic activity which has been carried, where the Herbal tablet formulation showed antilithiatic activity in the term inhibiting the formation of the calcium oxalate crystals. The crystallization process was initiated by adding calcium chloride (4 mol/L) and sodium oxalate (50 mol/L) solutions to artificial urine that had been prepared in a buffer with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 and 37 °C. These results showed that herbal tablet formulation contained calcium oxalate crystallization preventing properties. This property of plants may be an important in preventing the growth of renal lithiasis.

5. ACKNOWLEDGMENTS

The authors thanks the principal, Kamarajar college of pharmacy, Keerapalayam -608 602 Chidambaram –TK, cuddalore – DT for providing the necessary facilities to carry out the work.

I extend my thanks to all faculty members Dr.P.Veeramani, Mr. A. Bharathiraja, Mrs. B. sivaranjani, Mrs. M. Sudha, and the non-teaching staff for their valuable guidance and constant support.

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