

Phytochemical Investigation and Evaluation of In-vitro Antilithiatic Activity of *Sphaeranthus indicus* Roots

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ABSTRACT

Sphaeranthus indicus Linn. (Asteraceae) is widely used in *Ayurvedic* system of medicine to treat vitiated conditions of epilepsy, mental illness, hemicrania, jaundice, hepatopathy, diabetes, antilithiatic activity, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. There are reports providing scientific evidences for hypotensive, anxiolytic, neuroleptic, hypolipidemic, immunomodulatory, antioxidant, anti-inflammatory, bronchodilatory, antihyperglycemic and hepatoprotective activities of this plant. A wide range of phytochemical constituents have been isolated from this plant including sesquiterpene lactones, eudesmenolides, flavanoids and essential oil. A comprehensive account of the morphology, phytochemical constituents, ethnobotanical uses and pharmacological activities reported are included in this review for exploring the immense medicinal potential of this plant.

Keywords: *Sphaeranthus indicus* roots, urolithiasis, calcium oxalate, calcium phosphate, urinary stones.

INTRODUCTION

Urolithiasis is the word derived from Greek words *Ouron* (urine) and *lithos* (stone). Urolithiasis means formation of stone in the urinary system (calculi formed or located anywhere in the urinary system). [1] It comprises Nephrolithiasis, formation of stones in Kidney; Ureterolithiasis, formation of stones in the ureter, and cystolithiasis formation of stones in bladder.

Urinary tract stones

1. calcareous stones (calcium containing & radio-opaque) (75-90%)
 - a) Calcium oxalate (whewellite & weddellite)
 - b) Basic calcium phosphate
2. Non calcareous stones (non radio-opaque)
3. Struvite (Magnesium ammonium phosphate) (10-15%)
4. Uric acid (3-10%)
5. Cystine (0.5-1%)

About 80% of those with kidney stones are men. Men most commonly experience their first episode between 20-30 years of age, while for women the age at first presentation is somewhat later. Kidney stones typically leave the body in the urine stream, and a small stone comes in waves lasting 20 to 60 minutes. Other associated symptoms include: nausea, may pass without causing symptoms. If stones grow to sufficient size (usually at least 3 millimeters (0.1 in)) they can cause blockage of the ureter.

Typically vomiting, fever, blood in the urine, pus in the urine, and painful urination. Blockage of the ureter can cause decreased kidney function and dilation of the kidney. Most stones form due to a combination of genetics and environmental factors. Risk factors include being overweight, certain foods, some medications, and not drinking enough fluids. The diagnosis is usually based on symptoms, urine testing, and medical imaging. Blood tests may also be useful.

The development of urinary stones is most commonly related to;

- Decreased urine volume
- Increased excretion of stone-forming components
- Inadequate urine drainage, which may lead to stasis
- Decrease in urinary citrate levels leading to deposition of calcium
- Deficiency of vitamins A or C-these conditions can also lead to the "Hypertraid": hyperparathyroidism, hypercalcaemia, and hyperuricosuria.

Ureteral obstruction causes postrenal Zotemia and hydronephrosis (distension and dilation of the renal pelvis and calyces), as well as spasm of the ureter. This leads to pain, most commonly felt in the flank (the area between the ribs and hip), lower abdomen, and groin(a condition called renal colic). Renal colic can be associated with nausea, vomiting, fever, blood in the urine, pus in the urine, and painful urination. Renal colic typically comes in waves lasting 20-60 minutes, beginning in the flank or lower back and often radiating to the groin or genitals. The diagnosis of kidney stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies. Ultrasound examination and blood tests may also aid in the diagnosis.

Among ruminants, uroliths more commonly cause problems in males than in females; the sigmoid flexure of the ruminant male urinary tract is more likely to obstruct passage. Early-castrated males are at greater risk, because of lesser urethral diameter.³

Pelleted feeds may be conducive to formation of phosphate uroliths, because of increased urinary phosphorus excretion. This is attributable to lower saliva production where pelleted rations containing finely ground constituents are fed. With less blood phosphate partitioned into saliva more tends to be excreted in urine.(most saliva phosphate is fecally excreted).⁸

Oxalate uroliths can occur in ruminants, although such problems from oxalate ingestion may be relatively uncommon. Ruminant urolithiasis associated with oxalate ingestion has been reported. However, no renal tubular damage or visible deposition of calcium oxalate crystals in kidneys was found in yearling weather sheep fed diets containing soluble oxalate at 0.6% of dietary dry matter for about 100days.¹⁰

A person with recurrent kidney stones may be screened for such disorders. This is typically done with a 24 hours urine collection. The urine is analysed for features that promote stone formation. Calcium is one component of most common type of human kidney stones, calcium oxalate. Some studies suggest people who take calcium as a dietary supplement have a higher risk of developing kidney stones.

However, certain behaviors associated with frequent and binge drinking can lead to dehydration, which can in turn lead to the development of kidney stones. The American urological Association has projected that global warming will lead to an increased incidence of kidney stones in the United states by expanding the "kidney stone belt" of the southern United states.

People with lymphoproliferative/myeloproliferative disorders who were treated with chemotherapy developed symptomatic stones 1.8%of of the time in one study.

LITERATURE REVIEW OF PLANT

1. Sphaeranthus africana – Tanzania, Madagascar , Iran , Indian Subcontinent , China , Southeast Asia , northern Australia.
2. Sphaeranthus amaranthoides – Sri Lanka, Kerala, Tamil Nadu, Karnataka
3. Sphaeranthus angolensis – Angola
4. Sphaeranthus angustifolius – Madagascar
5. Sphaeranthus bullatus – Tanzania
6. Sphaeranthus chandleri – Uganda
7. Sphaeranthus confertifolius – Kenya
8. Sphaeranthus cristatus – Tanzania
9. Sphaeranthus epigaeus – South Africa
10. Sphaeranthus fischeri – Tanzania

OBJECTIVES OF PRESENT STUDY

Though the advances in modern medicines are significant, they remain an even increasing demand for herbal medicine. Herbal medicines as effective and potent medicine require evaluation by standard scientific methods so as to be validated for the treatment of diseases. The present patent laws have

increased the necessity to preserve the claims of these time-tested folk medicine. Thus it has become imperative that steps be taken to document components and activity of these medicinal plants. A systemic pharmacognostical, phytochemical and pharmacological evaluation of natural products form an intrinsic part of pharmacognosy. The present study is one such attempt to carry out.

- To extract roots of *Sphaeranthus indicus* plant by using Methanol
- To isolate the active principles present in extract by performing various chemical tests
- To investigate the *In – vitro* Antilithiatic activity of the Methanolic extract of the root at different concentrations.

PLANT PROFILE OF SPHAERANTHUS INDICUS

Common (Indian) Names

Sanskrit: Mahamundi, Mundi, Hapus,
Hindi, Bengali, Marathi, & Gujarati: bMundi, Gorkhmundi,
Telugu: Boddatarupa, Boddasoramu
Tamil: Kottak aranthai
Malyali: Mirangani
Riya: Murisa, Bokashungi
Punjabi: Ghundi, Khamadrus



Habitat: Common rabi weed found in rice fields.

Distributed through India, Srilanka, Africa and Australia. Related species *Sphaeranthus africanus* L. (Sanskrit – Sveta Hapusa; Malyali-Velutha adakkamaniyan)

Useful parts: Root, bark, leaves, flowers, and seeds.

Medicinal properties and uses

According to Ayurveda: Laxative digestive tonic, fattening, alterative, anthelminthic and alexipharmic.

Other: insanity

Tuberculosis

Indigestion

Bronchitis

Spleen diseases

Elephantiasis

Anaemia

Pain in uterus and vagina

Piles

Asthama

Leucoderma

Dysentery

Hemicrania etc.....

MATERIALS AND METHODS

COLLECTION OF PLANT MATERIAL: Plant material collected from rice crop fields of Narakoduru village, Roots are seperated and washed throughly with water dried and grinded to get coarse powder of roots. All the chemicals use for the experiment are analytical grade and were procured from National scientifics, Guntur.

EXTRACTION PROCEDURE

250gms of coarse powder was packed in a thimble made of filter paper which was then placed to the wider part of the extractor by drug praticles. Menstrum is poured into the wider part of the extractor to the equal heights of the siphon tube. Menstrum was placed in round bottomed flask and boiled continuously.

Preparation of Semi permeable membrane

The semi permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCL for overnight, which caused complete decalcification. Further, washed with distilled water and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Then the egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while& rinsed it with distilled water. Stored in refrigerator at a PH of 7-7.4.

Preparation of standard solution

A polyherbal formulation such as Cystone was selected and tablets were placed in absolute methanol for removing colour coating and were crushed into powder form. The power was dispersed into 100ml of distilled water and filtered. Filterate was used as positive control.

ANTILITHIATIC ACTIVITY OF METHANOLIC EXTRACT OF SPHAERANTHUS INDICUS

1)In-vitro Antilithiatic activity test by calcium oxalate dissolution method

Preparation of calcium oxalate by homogenous precipitation

1.47gm of calcium chloride dehydrate was dissolved in 100ml distilled water and 1.34gm of sodium oxalate was dissolved in 100ml of 2N H₂SO₄. Both were mixed equally in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate was freed from traces of sulphuric acid by ammonia solution: washed with distilled water and dried at a temperature 60degree C for 2 hrs.

EXPERIMENT PROTOCOL

Control	1ml(1mg/ml) of calcium oxalate+1ml of water
Test	1ml of calcium oxalate+1ml(10mg/ml)SIME
Test	1ml of calcium oxalate+1ml(20mg/ml)SIME
Test	1ml of calcium oxalate+1ml(30mg/ml)SIME
Test	1ml of calcium oxalate+1ml(40mg/ml)SIME
Standard	1ml of calcium oxalate+1ml(400mg/ml)cystone

SIME : *Sphaeranthus indicus* Methanolic extract

All the models were allowed to suspend in conical flasks containing 100ml of 0.1MTris buffer. All the flasks were subjected to incubated for three days. After three days the membranes were taken out of the flask and content of each membrane was collected in different test tubes. 2ml of 1N sulphuric acid was added to each test tube and titrated with 0.9494N KMNO₄ till the colour disappears. 1ml of 0.9494N KMnO₄ is equivalent to 0.1898mg of calcium.

The amount of undissolved calcium oxalate is subtracted from the total quality used in the experiment in the beginning, to know much quantity of calcium oxalate actually test substances could dissolve.

2) IN-VITRO ANTILITHIATIC ACTIVITY TEST BY CALCIUM PHOSPHATE DISSOLUTION METHOD

Preparation of calcium phosphate by homogenous precipitation

1.47gm of calcium chloride dehydrate was dissolved in 100ml distilled water and 1.42gm Of disodium hydrogen phosphate was dissolved in 100ml of 2N sulphuric acid. Both were Mixed equally in a beaker to precipitate out calcium phosphate with stirring. The resultant Calcium phosphate was freed from traces of

sulphuric acid by ammonium solution; washed with distilled water and dried at temperature 60 degree C for 2hrs.

EXPERIMENTAL PROTOCOL

Control	1ml(1mg/ml)of calcium phosphate+1ml of water
Test	1ml of calcium phosphate+1ml(10mg/ml)SIME
Test	1ml of calcium phosphate+1ml(20mg/ml)SIME
Test	1ml of calcium phosphate+1ml(30mg/ml)SIME
Test	1ml of calcium phosphate+1ml(40mg/ml)SIME
standard	1ml of calcium phosphate+1ml(400mg/ml)cystone

SIME: Sphaeranthus indicus Methanolic extract

All the models were allowed to suspended in conical flasks containing 100ml of 0.1MTris buffer. All the flasks were subjected for 3 days. After 3 days the membranes were test out of the flask and content of each membrane was collected in different test tubes. 4ml of 1N sulphuric acid 3ml of molybdate-sulphuric acid reagents. 1ml of reducing solution were added and kept a side for 2hrs. Colour change from dark pink to colour less was observed after 2hrs. Change in colour intensity was measured against 620nm spectrophotometrically. Concentration of undissolved calcium was determined from standard calibration curve of calcium phosphate by using measured absorbance readings.

$$\%inhibition = \{1 - [si/sc]\} \times 100$$

Where; **si**: slope of graph in the presence of inhibitor (plant extract),
Sc: slope of graph without inhibitor (control)

RESULTS AND DISCUSSTION

Phytochemical Investigation

Methanolic extract of fruit has shown the presence of Carbohydrates and Fats, Proteins and Vitamins.

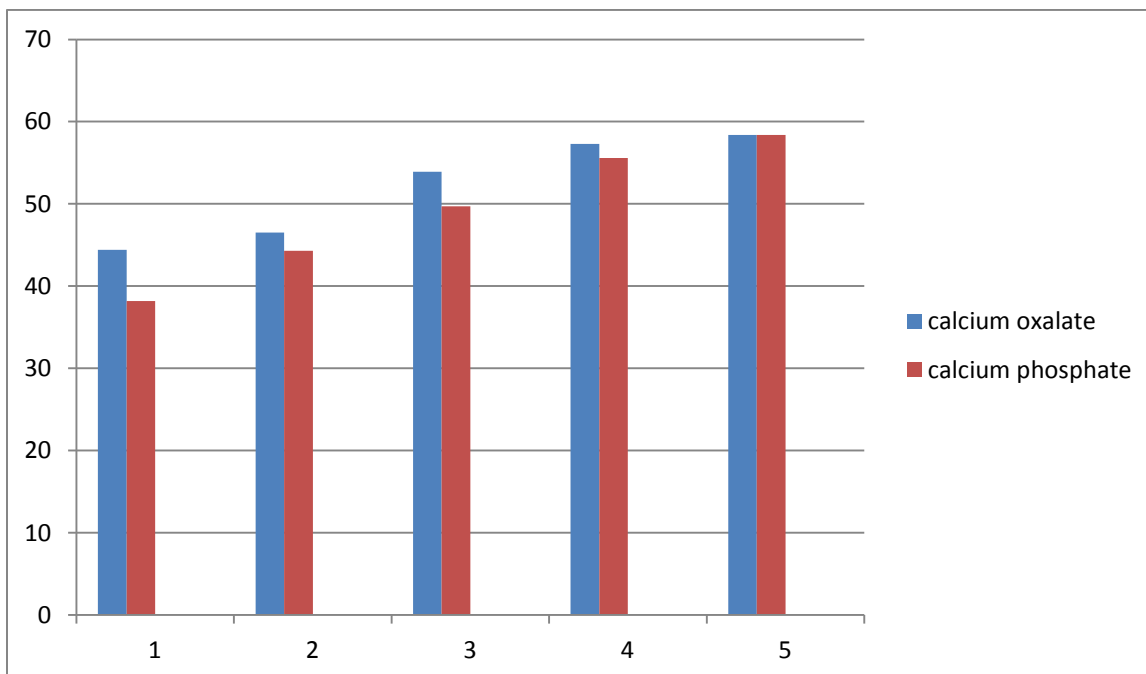
PHYTOCHEMICAL INVESTIGATION

Chemical test for the Methanolic extract of Sphaeranthus indicus

NAME OF THE TEST	METHANOLIC EXTRACT
Triterpenes	
A. Salkowski Test:	+
B. Liebermann – Buchard's Test:	+
C. Ischugajiu Test:	+
D. Brickorn and Brinar Test:	+
Saponins:	
A. Foam test:	+
B. Haemolysis Test:	+
Alkaloids:	
A. Mayer's Test:	+
B. Dragendroff's Test:	+
C. Wagner's Test:	+
D. Hager's Test:	+
Carbohydrates:	
A. Molisch's Test:	+
B. Fehling's Test:	+
C. Benedict's Test:	+
D. Barfoed's Test:	+

Effect of Methanolic extract was statistically equal to the effect of standard drug being used for dissolving the existing renal stone. Methanol extract of four concentrations were taken for experimental purpose (10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml). dissolution of crystals were less for Methanolic extract of 10mg/ml and 20mg/ml concentrations. Whereas the extract of 30mg/ml and 40mg/ml has shown almost similar dissolution of crystals as that of standard Cystone of 400mg/ml.

Treatment Groups	Calcium oxalate Absorbance At620nm	Calcium oxalate %Dissolution	calcium phosphate Absorbance At620nm	calcium phosphate %Dissolution
Control	1.562	0.0	1.562	0.0
methanolic extract10mg/ml	0.472	41.4±0.02	0.465	38.2±0.02
methanolic extract20mg/ml	0.556	46.5±0.02	0.532	44.3±0.02
methanolic extract30mg/ml	0.598	53.9±0.03	0.574	49.7±0.02
methanolic extract40mg/ml	0.643	57.3±0.03	0.629	55.69±0.02
Standard	0.650	58.4±0.02	0.650	58.4±0.02



CONCLUSION

The above results concluded that the plant is available as a weed in rice crop. We can get rid of weeds from the crop at the same time the plant is beneficial for treating various diseased conditions in human beings. According to the knowledge of the tribal people the root decoction of Bodatharamu has an amazing reduction in stone size. Based upon the results of present experiment conducted; SIME 40mg/ml, 50mg/ml, shown almost similar dissolution in calcium oxalate crystals in *-vitro*. Whereas SIME 10mg/ml, 20mg/ml has shown less dissolution. Thereby it was concluded that it is an amazing plant acts against various pathological conditions of human being. Further studies to be conducted for more information is under process.

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