

## EVALUATION OF IN-VITRO ANTI-UROLITHIASIS POTENTIAL OF AERIAL PARTS OF *GOMPHRENA GLOBOSA LINN*

P. Hemadharsni<sup>\*1</sup>, Mohanamuruga J.<sup>2</sup>, Mukesh Kumar S.<sup>3</sup>, Navaneetha Krishnan M.<sup>4</sup>, Rajesh R.<sup>5</sup>, Dr. S. Kannan<sup>6</sup>, Dr. B. Sangameswaran<sup>7</sup>

<sup>1</sup>Assistant Professor, Department of Pharmacology, SSM College of Pharmacy, The Tamil Nadu Dr. M.G.R. Medical University.

<sup>2,3,4,5</sup>B.Pharm Final Year Research Scholars, SSM College of Pharmacy, The Tamil Nadu Dr. M.G.R. Medical University.

<sup>6</sup>Professor & HOD Department of Pharmacology, SSM College of Pharmacy, The Tamil Nadu Dr. M.G.R. Medical University.

<sup>7</sup>Principal, SSM College of Pharmacy, The Tamil Nadu Dr. M.G.R. Medical University.

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\*Corresponding Author: P. Hemadharsni

Assistant Professor, Department of Pharmacology, SSM College of Pharmacy, The Tamil Nadu Dr. M.G.R. Medical University.

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### ABSTRACT

Effective preventative and treatment measures are required due to the rising prevalence of kidney stones, also known as urolithiasis. Despite their ability to reduce symptoms, pharmaceutical therapies are frequently linked to undesirable side effects. Because plant therapies are safe, effective, and have fewer side effects, they are highly recommended in Ayurveda and Unani medicine for the treatment of urolithiasis. Numerous bioactive substances found in plants have the ability to decrease the formation of stones, boost urine production, and dissolve existing stones. *Gomphrena globosa* (L.), also referred to as Globe Amaranth, is one of the historically used herbs whose potential as a treatment for kidney problems has drawn attention. The potential of herbal treatments, including, *Gomphrena globosa* (L.) as alternatives to traditional medications is highlighted. Within the context of traditional and modern treatment approaches for urolithiasis, by highlighting a promising area for further research and clinical practice, this study aims to advance our understanding of plant remedies, particularly the therapeutic benefit of *Gomphrena globosa* (L.), for kidney stone prevention and management.

**KEYWORDS:** Urolithiasis, *Gomphrena globosa* (L.), Anti Urolithiatic.

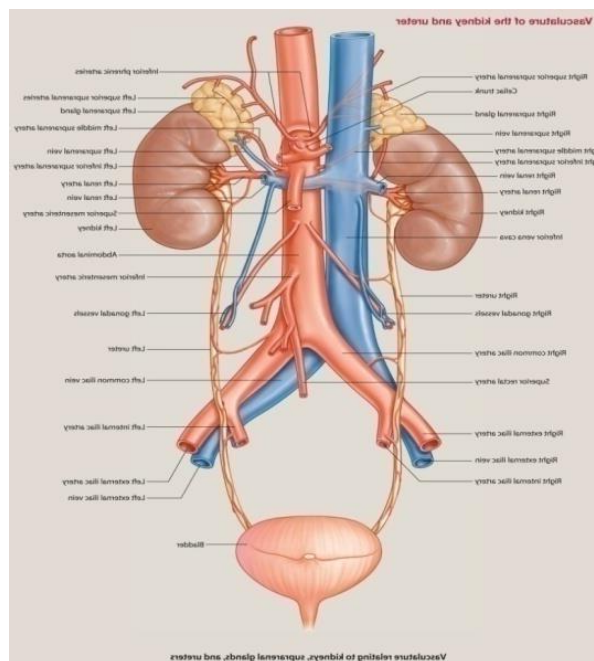
## INTRODUCTION

Traditional systems of medicine, including Ayurveda and Unani, have been commonly practiced because of population expansion, poor drug availability, excessive treatment charges, side effects, and resistance to existing drugs. Plant-based drugs are being used more in developing countries because modern life-saving drugs are out of reach for three-fourths of the third world's population. India, which is one of the 12 canters of biodiversity in the world, is also blessed with large numbers of medicinal plants, numbering more than 2,50,000 species and more than 80,000 medicinal plants. Forests of India are the major source of medicinal and aromatic plants for drug and perfumery industries.

Plants, especially of Ayurveda, can be a source of biologically active molecules and lead structures for developing modified derivatives having improved activity or less toxicity. Green plants produce and store biochemical products, most of which are extractable and utilized as chemical feed stocks or raw materials for scientific enquiry. Invariably, though, a continuous supply of source material tends to become problematic owing to causes such as environmental fluctuations, cultural habits, geographical distribution, cost of labor, choice of higher plant stock, and pharmaceutical overexploitation.

Single active compound works in mixtures of traditional medicine and are most important, because isolation and identification of active principles and determination of drug mechanisms are of supreme importance.<sup>[1]</sup>

## ANATOMY OF URINARY SYSTEM

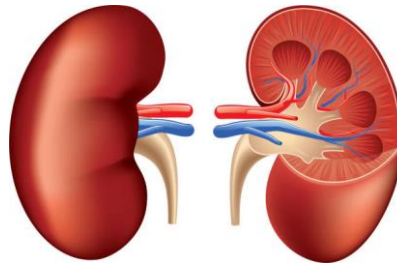


**Figure 1: Urinary System.**

The main organs of the urinary system are:

- A Pair of kidneys (blood-filtering organs).
- Ureters (ducts that connects the kidneys to bladder)
- A bladder (an organ that holds pee).
- A urethra (a tube connected to the bladder that allows pee to leave your body).

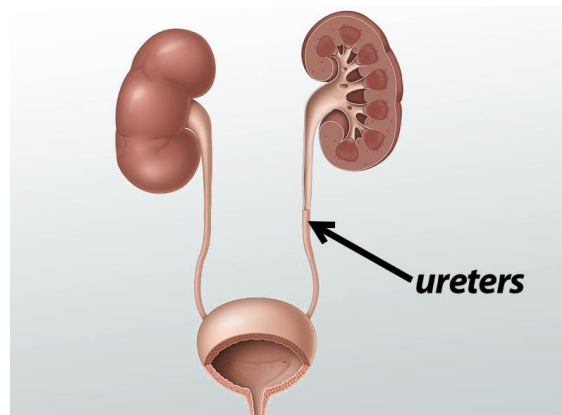
## KIDNEYS



**Figure 2: Structure of kidneys.**

In the urinary system, the kidneys—which are found on the spine—are essential organs that filter waste, fluids, and electrolytes, maintain fluid balance, control blood pressure, and produce hormones that support healthy bones and the creation of red blood cells

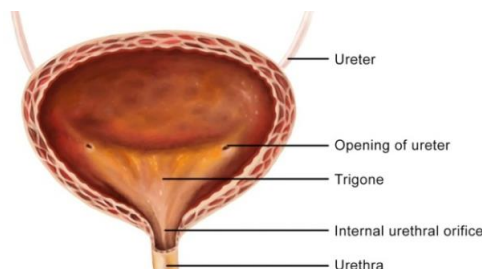
## URETERS



**Figure 3: structure of ureters.**

Urine is transported from the kidneys to the bladder via Ureters. The two Ureters in each human are around 8 to 10 inches long. Urine is moved downward by the contraction of their smooth muscles. Three layers make up the Ureters' walls Fibrous connective tissue, which gives stability and strength, is the first layer. The smooth muscles in the middle layer contract to force urine into the bladder. Inner layer Protective epithelial membranes.<sup>[2]</sup>

## BLADDER

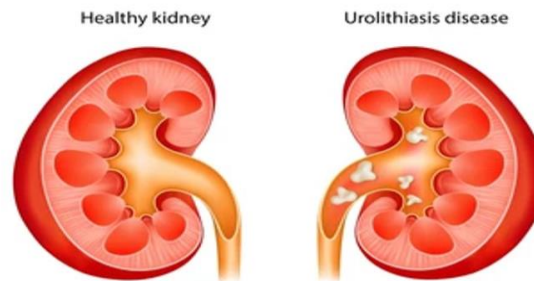


**Figure 4: Bladder.**

The bottom portion of your abdomen, or belly, contains the bladder. The bladder is held in place by bands of tissues called ligaments that attach it to your hip bone (pelvis) and other organs. It sits between the rectum in the rear and the pubic bone in the front of men. It sits in front of the uterus and vagina in women.

## UROLITHIASIS

Urolithiasis, also known as Nephrolithiasis, is a common illness that affects 2–15% of people worldwide<sup>[3]</sup>. Numerous risk factors and lifestyle choices can have an impact on this condition, which is characterized by the development of calcifications in the urinary system, usually in the kidneys or bladder.<sup>[4]</sup>



**Figure 5: Different between normal and affected kidney.**

Kidney stones are influenced by various factors, including age, gender, family history, climate, diet, water quality, genetics, and co-morbidities. The incidence and prevalence of urolithiasis vary geographically. For example, the prevalence in mainland China was 7.54% between 1990 and 2016, 5-10% in Europe before 2011, 8.8% in North America between 2007-2010, and 5.7% in Iran in 2005. In India, around 1% of emergency admissions are due to renal colic and complications of renal stones, with a prevalence of approximately 12%, higher in the northern regions at 15%. Kidney stones, particularly calcium oxalate stones (60%), often recur, with about 98% of patients developing another stone within 25 years. Stones form through the crystallization of substances in urine, and if they cannot pass through the Ureters, they cause pain and potential obstruction.<sup>[5-12]</sup>

## FORMATION OF KIDNEY STONE

Kidneys maintain the body's balance of water, minerals, and salts, producing urine as a result of this filtering process. Kidney stones (Nephrolithiasis or urolithiasis) form when substances like calcium, oxalate, and phosphate become overly concentrated in urine, leading to crystal formation and accumulation. About 80% of kidney stones are made of calcium oxalate, often mixed with calcium phosphate. Other types, like uric acid, struvite, and cystine stones, make up the remaining 20%. Factors like super saturation of calcium oxalate and nucleation, where ions combine into solid crystals, increase the risk of stone formation. Additionally, other factors such as inhibitors of crystallization and the urothelial surface properties also play a role in stone formation.<sup>[13]</sup>

## TYPES OF KIDNEY STONES

Kidney stones have been associated with an increased risk of chronic kidney diseases and that leads to end-stage of renal failure cardiovascular diseases diabetes, and hypertension.<sup>[14]</sup> Kidney stones are made of different types of crystals.

- CALCIUM STONES [Calcium oxalate, calcium phosphate or a combination of calcium oxalate and calcium phosphate]
- URIC ACID STONES
- STRUVITE STONES
- CYSTINE STONES

### CALCIUM STONE

Kidney stones are primarily composed of calcium and oxalate. Conditions like hypercalciuria (excessive calcium in the urine) and hyperoxaluria (excessive oxalate in the urine) contribute to the formation of calcium oxalate stones. High oxalate intake from food, drinks, or excessive Vitamin C consumption, which is metabolized to oxalate, increases stone formation risk.<sup>[15]</sup>



**Figure 6: Calcium oxalate stone.**

Additionally, factors like increased bile salt and fatty acid levels promote oxalate absorption, leading to more urinary oxalate and calcium oxalate stones. Chronic diarrhea can cause metabolic acidosis, reducing urinary citrate and magnesium. Calcium phosphate stones are linked to urinary tract infections, Renal Tubular Acidosis (RTA), and hyperparathyroidism. In acidic urine (pH <5.5), uric acid crystals form, and calcium oxalate stones can develop as calcium layers around these crystals. High urinary oxalate levels are more influential in stone formation than high calcium levels.<sup>[16]</sup>

### URIC ACID STONES

Uric acid stones form when urine pH is greater than 7.2 and ammonia is present, and bacteria that create urea degrade into ammonia, bicarbonate, and carbonate. Uric acid, a metabolite of purine metabolism, is the identical crystal that causes gout.

A purine rich diet beef, poultry, pork, fish, and organ meats—may increase uric acid production. Uric acid stones occur when uric acid is too high or the urine is too acidic. Gout, diabetes, and chemotherapy can raise the risk of these stones.<sup>[17]</sup>



**Figure 7: Uric acid stone.**

### CYSTINE STONES

Cystine or Cystinuria is an Autosomal recessive disorder that causes impaired renal tubular re absorption of cystine, ornithine, lysine and argentine. This leads to increased urinary excretion of these compounds, but the only one that forms stone in the cystine.

## STRUVITE STONES

Struvite urinary stones, also known as "infection stones" or "triple phosphate stones," are composed of magnesium ammonium phosphate. The term "triple phosphate" comes from early chemical analyses showing the presence of calcium, magnesium, ammonium, and phosphate. Carbonate ions were also commonly found thought to be associated with calcium as calcium carbonate (CaCO<sub>3</sub>). Modern crystallographic analysis reveals that human struvite stones are a mixture of struvite and carbonate-apatite. While calcite (CaCO<sub>3</sub>) is rare, it may be more abundant in some cases.<sup>[18]</sup>

### Risk Factors

Urolithiasis is influenced by a complex interplay of genetic, environmental, and lifestyle factors. The primary risk factors for kidney stone formation include:

**Dehydration:** Insufficient fluid intake leads to concentrated urine, which promotes the crystallization of salts and minerals. Persons who do not drink enough water, or sweat excessively, are at higher risk of developing stones.

**Dietary Factors:** Diet plays a significant role in stone formation. High intake of salt, animal proteins and oxalate-rich foods (such as spinach, beets, and nuts) increases the risk of calcium oxalate stone formation. A diet high in purines (found in red meat, organ meats, and seafood) can contribute to uric acid stones.

**Obesity:** Obesity increases the risk of stone formation due to altered urinary composition, including higher urinary calcium and oxalate levels, which promote stone formation.

**Genetic Factor:** Family history plays a significant role in the development of kidney stones. Individuals with a first-degree relative who has had stones are more likely to develop stones themselves.

**Medications:** Medications, such as diuretics, calcium-based antacids, and protease inhibitors, can increase the risk of stone formation.

**Infections:** Recurrent urinary tract infections (UTIs), particularly those caused by urea producing bacteria, can lead to the formation of struvite stones, which are typically larger and more difficult to pass.<sup>[19]</sup>

## SYMPTOMS

Kidney stone causes a blockage, or it moves into the Ureters, it may cause some of the following symptoms:

- Severe pain or aching in the back on one or both sides
- Sudden spasms of excruciating pain (renal or uteri colic); this usually starts in the back below the ribs, before radiating around the abdomen and sometimes to the groin and genitalia
- Bloody, cloudy or smelly urine
- Feeling of being sick
- A frequent urge to urinate or a burning sensation during urination
- Fever and chills, nausea and vomiting

These can also be symptoms of a urinary tract infection or cystitis, which is much more common than kidney stones in young women. Kidney stones are usually passed out of the body within 48 hours, but attacks can sometimes last for over 30 days.

## DIAGNOSIS

Diagnosis of kidney stones involves several tests:

- **Blood test:** Measures calcium or uric acid levels to monitor kidney health and identify underlying conditions.
- **Urine test:** A 24-hour collection can detect high levels of stone-forming minerals or low levels of protective substances.
- **Imaging:** High-speed CT scans can detect even tiny stones, while abdominal X-rays are less effective. Ultrasound is a quick, non-invasive alternative for diagnosing kidney stones.
- **Analysis of passed stones:** Stones passed through urine are collected using a strainer and analyzed in a lab to determine their composition.<sup>[20]</sup>

## HERBAL MEDICINE IN UROLITHIASIS MANAGEMENT

Systems of traditional medicine such as Ayurveda, Traditional Chinese Medicine (TCM), and indigenous medicine have been recognized for employing plant-based therapies in the prevention and treatment of kidney stones. Such therapies are appreciated for their potential efficacy, safety, and fewer side effects than those of conventional drugs. Herbs have been discovered to act through a variety of mechanisms such as<sup>[21]</sup>:

- Urine alkalisation and enhanced urinary output
- Reduction of stone formation by modulating crystal growth
- Antioxidant and anti-inflammatory actions
- Antimicrobial activity against infection stones
- Solubilising existing stones

## PREVENTION

### PHYSICAL HEALTH

Maintaining a normal BMI is recommended for kidney stone prevention. While the benefits of physical activity are well-known, their specific role in preventing kidney stones is still unclear. Dehydration during and after intense physical activity is a concern, and proper fluid compensation is crucial. Working in high temperatures may lead to dehydration, so staying hydrated is important. Avoiding smoking is also beneficial for overall health.

### Dietary Management

- **Calcium stones:** Consume 1000–1200 mg of dietary calcium daily and limit sodium intake.
- **Calcium oxalate stones:** Limit oxalate-rich foods (e.g., spinach, soy, nuts, beets, and raspberries).
- Drink more than 3 litres of water to keep urine clear.
- Avoid vitamin C and D supplements.
- Limit animal protein intake while increasing plant proteins.
- Increase citrus fruits (grapefruit, lemon, lime) in the diet to help prevent stones<sup>[22]</sup>.

## PLANT PROFILE

### DESCRIPTION

*Gomphrena globosa* Linn is commonly referred to as the 'Bachelor's button' / Globe Amaranth / kenop flower and has been identified in a variety of traditional medicine systems for the treatment of various human diseases.



Figure 8: *Gomphrena globosa* (L.)

#### SYNONYMS

*Gomphrena globosa* L.

*Gomphrena celosioides*

*Gomphrena serata*

#### TAXONOMICAL CLASSIFICATION

Kingdom: Plantae

Subkingdom: Tracheobionta

Super Division: Spermatophyta

Division: Magnoliopsida

Class: Magnoliopsida

Subclass: Caryophyllidae

Order: Caryophyllales

Family: Amaranthaceae

Genus: *Gomphrena* L.

Species: *G. globosa*

#### PLANT INFORMATION

**Common Names:** Globe Amaranth, Bachelor's Button.

**Tamil Name:** Vadamalli,

**Native Regions:** North America, South America, Myanmar, India, Brazil, Panama and Bangladesh.



Figure 9: Aerial parts of *Gomphrena globosa* (L.)



**Growth Habit:** Tropical annual plant

**Plant Height:** Grows 1-2 feet tall

**Flowers:** Rounded, ball-like shape, occurring in colours such as dark red, Purple, White or Pink.

Blooms continuously through summer and early fall.

**Leaves:** Opposite, oblong, 5-10 cm long, sub-sessile

**Flower Heads:** 2.5-3.7 cm in diameter, pinkish purple, globose with two leafy

Bracts terminating the branches.

**Flowers:** Individual flowers within heads are inconspicuous;

Papery bracts are stiff and colourful.

**Fruit:** Oblong, ovoid capsule with brown smooth shining seeds.<sup>[23]</sup>

## MATERIALS AND METHODS

### Materials

- Calcium chloride
- Sodium oxalate
- Sulphuric Acid
- Tris - Buffer
- Cystone
- Sodium chloride
- Microscope

### METHODS

- Ethanolic solvent extraction
- Phyto chemical tests
- Calcium oxalate method

### PLANT MATERIAL

The aerial parts of *Gomphrena globosa L.* were collected from the local area of Komarapalayam in the month of October, Namakkal district, Tamilnadu, India. The plant material was authenticated by **Dr. P. RADHA; Research Officer (botany) Sci II & Vc; Siddha Medicinal Plants Garden / Mettur Dam, Tamilnadu – 636401.** And a voucher specimen {G291124202G} was deposited at the, SSM College of Pharmacy, Erode (638312) Tamilnadu, India.

### EXTRACTION

The ethanolic extract of *Gomphrena globosa L.* was prepared by maceration. The plant's aerial parts (flowers, leaves, and stems) were collected, washed, and dried at temperatures below 40°C, then ground into a coarse powder. A known amount of powder was mixed with 70% ethanol in a 3:1 ratio and left to macerate at room temperature for 72 hours, with gentle shaking every 24 hours. After 72 hours, the mixture was filtered using what man filter paper to separate the solid plant material from the liquid extract. The ethanol was then evaporated, and the final extract was stored in a cool, dry place.<sup>[24]</sup>

**PRELIMINARY PHYTO CHEMICAL ANALYSIS TEST**

The aerial parts of *Gomphrena globosa L.* was subjected to systematic Phyto chemical screening by successively extracting with ethanol and the extracts were subjected for Phyto chemical investigation by qualitative chemical identification tests.

**DETECTION OF ALKALOIDS**

- a) **Dragendorff Test:** 1-2 ml of Dragendorff's reagent was added to a few ml of the extract. A reddish- brown or creamy white precipitate indicated the presence of alkaloids, while the absence of such precipitate indicated a negative result.
- b) **Mayer's Test:** 1ml of the extract was added with 1 ml of Mayer's reagent. White yellow colour precipitate indicates the presence of alkaloids.

**DETECTION OF CARDIAC GLYCOSIDES**

**Keller-Killani Test:** 1ml of filtrate was mixed with 1.5 ml of glacial acetic acid, 1 drop of 5% ferric chloride, and concentrated H<sub>2</sub>SO<sub>4</sub> (added along the side of the test tube). A blue- colour solution in the acetic acid layer indicated the presence of cardiac glycosides.

**DETECTION OF PROTEINS AND AMINO ACIDS**

- a) **Ninhydrin Test:** 2mL of filtrate was mixed with 2 drops of Ninhydrin solution. A purple-colour solution indicated the presence of amino acids.
- b) **Biuret Test:** 1 ml of the extract was treated with 4% NaOH and few drops of CuSO<sub>4</sub> solution. Formation of purple violet colour indicates the presence of proteins.

**DETECTION OF FLAVONOIDS**

- a) **Lead Acetate Test:** 1mL of plant extract was added to a few drops of 10% lead acetate solution. A yellow precipitate indicated the presence of flavonoids.
- b) **Ferric Chloride Test:** A few drops of 10% ferric chloride solution were added to an aqueous extract. A green precipitate indicated the presence of flavonoids.

**DETECTION OF PHENOLIC COMPOUNDS**

**Iodine Test:** 1mL of extract was mixed with a few drops of diluted iodine solution. A transient red colour indicated the presence of Phenolic compounds.

**DETECTION OF TANNINS**

- a) **Braymer's Test:** 1mL of extract was mixed with 3mL of distilled water, followed by 3 drops of 10% ferric chloride solution. A blue-green colour indicated the presence of tannins.
- b) **Gelatin Test:** 1 ml of extract was added with 1% gelatin solution containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

**DETECTION OF PHYTOSTEROLS**

**Salkowski's Test:** The extract was mixed with a few drops of concentrated H<sub>2</sub>SO<sub>4</sub>, shaken well, and allowed to stand. A red colour in the lower layer indicated the presence of Phyto sterols.

### DETECTION OF CARBOXYLIC ACIDS

**Effervescence Test:** 1mL of plant extract was mixed with 1 ml of sodium bicarbonate solution. The appearance of effervescence indicated the presence of carboxylic acids.

### DETECTION OF COUMARINS

**Naoh Test:** Plant extract was mixed with 10% NaOH and chloroform. A yellow colour indicated the presence of Coumarin.

### DETECTION OF QUINONES

**Conc. HCL TEST:** 2 ml of extract was added with conc. HCL. A green colour indicates presence of quinine.<sup>[25,26,27]</sup>

### EVALUATION OF ANTI UROLITHIATIC ACTIVITY

#### In-vitro anti-urolithiatic activity by aggregation assay method

The in-vitro anti-urolithiatic activity of *Gomphrena globosa L.* was studied by the aggregation assay method. Calcium oxalate monohydrate (Coax) crystals were synthesized by the combination of calcium chloride (50mmol/L) and sodium oxalate (50mmol/L) and equilibrating it in a water bath at 60°C. The crystals were cooled and suspended in Tris buffer of pH 6.5. Different concentrations of the ethanolic extract of *Gomphrena globosa L.* (10, 50, 100, 250, and 500 µg/ml) were added to the crystal solution and incubated at 37°C for 24 hours. Cystone (500 µg/ml) was used as the standard. Aggregation activity was measured by turbidity using a UV spectrophotometer at 620nm.<sup>[28]</sup> The percentage inhibition was calculated as: **% inhibition = [(OD control - OD test) / OD control] x 100.**

### RESULT AND DISCUSSION

#### Plant Extraction

The Plant extract was done by Maceration method using ethanol as solvent. Obtained plant extract was green viscous, semi solid in nature. The Percentage yield value of the extract was 16.22 %.

#### Preliminary Phytochemical Test Result

Preliminary Phyto chemical test of the ethanolic extracts revealed the presence and absence of different primary and secondary metabolites.

Aerial parts of *Gomphrena globosa L.* were found to contain the presence of Alkaloids, flavonoids, Phenolic compounds, phytosterol, and Coumarin.

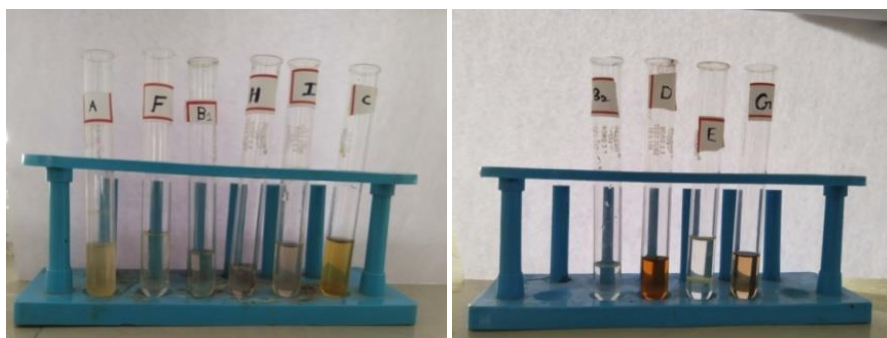


Figure 11: Results of the phyto chemical test.

**Table 1: Results of the preliminary phyto chemical test of eegg plant extract.**

S. No.	Constituents	Test Name	Report
1.	Alkaloids	Dragendorff's test Mayer's test	(+ ve) (- ve)
2.	Cardiac Glycoside	Killer killani test	(- ve)
3.	Proteins and Amino Acid	Ninhydrin test Biuret test	(- ve) (- ve)
4.	Flavanoids	Ferric chloride test Lead acetate test	(+ ve) (+ ve)
5.	Phenolic Compounds	Iodine test	(+ ve)
6.	Tannin	Braymer's test Gelatin test	(- ve) (- ve)
7.	Phyto Streols	Salkowski's test	(+ ve) [Presence of red layer]
8.	Carboxylic Acid	Effervescence test	(- ve) [No effervescence found]
9.	Coumarin	NAOH test	(+ ve)
10.	Quinones	Conc. HCL TEST	(- ve)

- The +ve symbol indicates the {positive}
- The -ve symbol indicates the {negative}

**Discussion:** The results revealed the presence of medically active compounds in the plant extract. Preliminary Phyto chemical studies confirmed the purity of the drug. The Phyto chemical investigation of the extract showed that the presence of Alkaloids, Flavonoids, Phenolic Compounds, Phyto sterol and Coumarin.

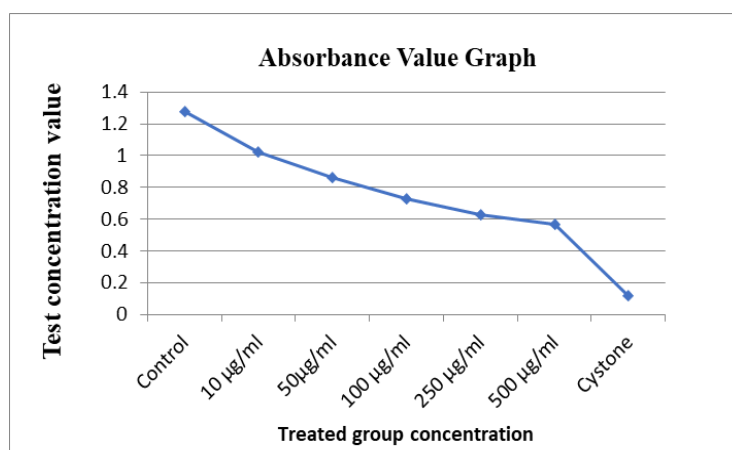
#### IN-VITRO ANTI-UROLITHIATIC ACTIVITY BY AGGREGATION ASSAY METHOD REPORT

**RESULT:** The results of in vitro anti urolithiatic activity of *Gomphrena globosa L.* by calcium oxalate method were given in the table no: 2 and 3 and figure no: 12 and 13.

#### A) Meaurment of UV absorbance value of the plant extract of eegg {Aerial parts of *Gomphrena globosa (L.)*}

**Table no.2: Shows the absorbance value of plant extract concentration of eegg with standard drug cystone.**

S. No.	Treated Sample	Optical Density Value [OD]			Mean Value
1.	Control	1.297	1.258	1.274	1.276
2.	10 µg/ml	1.012	1.026	1.029	1.022
3.	50µg/ml	0.839	0.870	0.807	0.859
4.	100 µg/ml	0.691	0.751	0.734	0.725
5.	250 µg/ml	0.612	0.638	0.612	0.630
6.	500 µg/ml	0.545	0.571	0.592	0.569
7.	Cystone	0.136	0.114	0.111	0.120

**Figure 12: Shows the graphical value for the absorbance value of eegg with standard drug cystone.**

**Discussion:** The formation of Calcium oxalate crystals was followed after mixing solution of Calcium chloride and Sodium oxalate without or with plant extract which served as a control and test, respectively. Measuring of Optical density through UV Spectrophotometer.

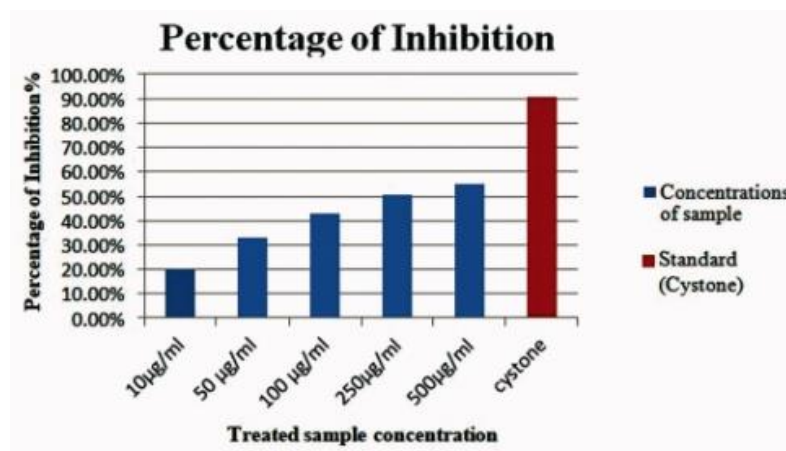
#### B) Percentage inhibition of calcium oxalate stones in plant extract of eegg with standard drug cystone

**Table no.3:** Shows the percentage inhibition value of plant gextract of eegg with standard drug cystone.

S. No	Treated sample	Percentage of Inhibition (%)
1.	10 µg/ml	19.52 %
2.	50µg/ml	33.07 %
3.	100 µg/ml	42.91 %
4.	250 µg/ml	50.39 %
5.	500 µg/ml	55.19 %
6.	Cystone	90.55 %

**Discussion:** The standard drug (Cystone 500µg/ml) achieved **90.55%**, the highest concentration [500µg / ml] extract achieved **55.19%**, at the concentration [250µg / ml] the extract achieved **50.39%**, at the concentration [100µg / ml] the extract achieved **42.91%**, at the concentration [50µg / ml] the extract achieved **33.07%**, And the lower concentration [10µg / ml] extract achieved **19.52 %** and the control achieved 0% of inhibition.

The plant extract exhibits inhibitory activity of the calcium oxalate crystals as the plant extract becoming more effective when the concentration level increases.



**Figure 13:** Shows graphical view of Percentage inhibition value of EEGG with standard drug Cystone in aggregation method.

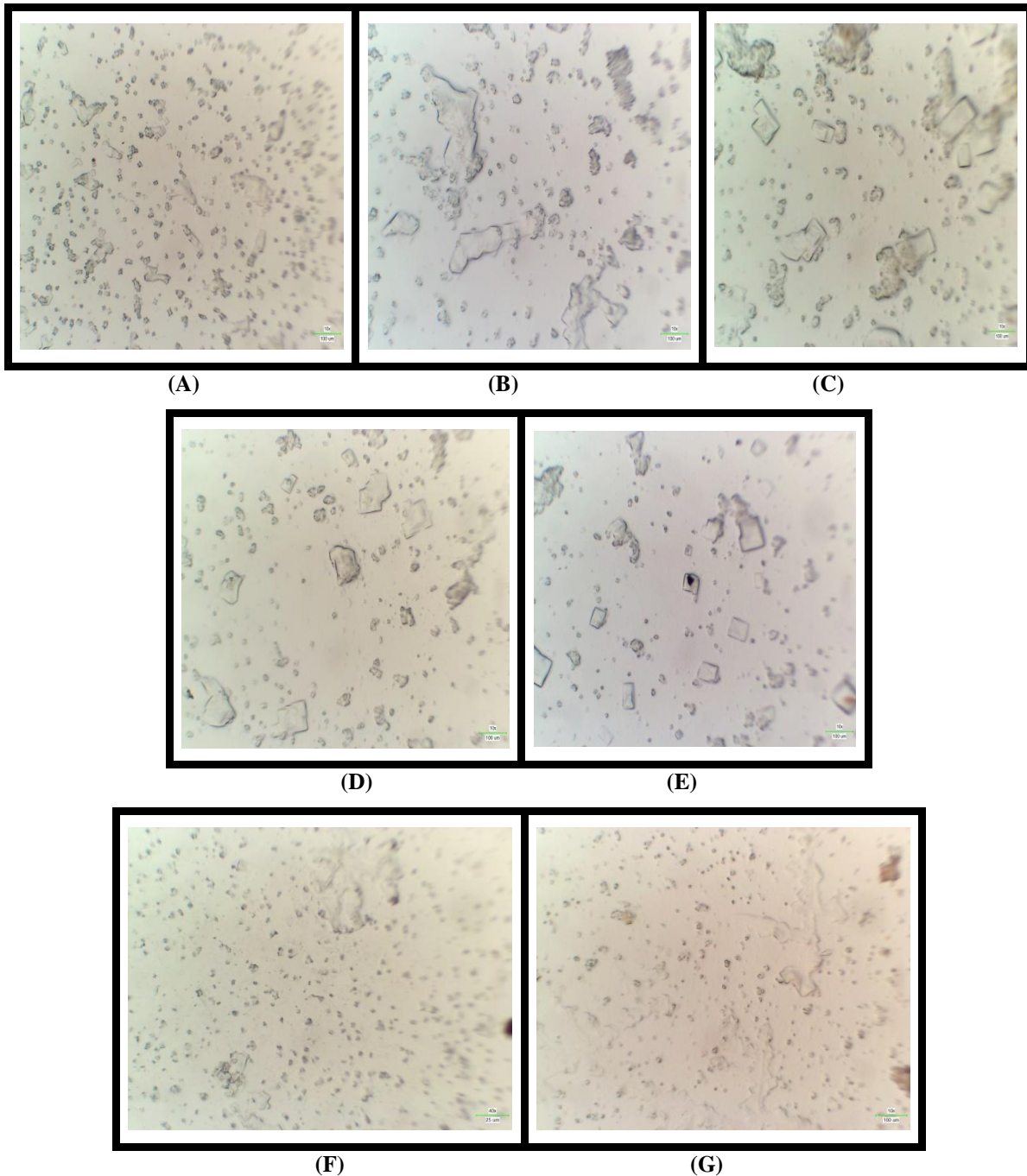
**Discussion:** Different dose of ethanolic extracts from lower to higher concentration had been prepared to evaluate the effect. Cystone was found to be more effective in anti urolithiatic activity, so it is taken as standard drug.

The Anti urolithiatic activity is done by aggregation method. The result indicates that the concentration of extract increased, the percentage inhibition also increased. The result demonstrating its potential of anti urolithiatic activity as compared with standard drug (Cystone 500 µg/ml).

The extract has an anti urolithiatic activity as compared to control but the extract has less potency as compared with standard drug Cystone.

**MICROSCOPE IMAGE OF THE CALCIUM OXALATE CRYSTALS**

**RESULT:** Microscopic images of the different concentration and standard drug results also given in figure no: 14



**Figure 14: Observed microscopic image of the calcium oxalate crystals at 10 x magnification.**

**a) Control b) 10  $\mu\text{g/ml}$  c) 50  $\mu\text{g/ml}$  d) 100  $\mu\text{g/ml}$  e) 250  $\mu\text{g/ml}$  f) 500  $\mu\text{g/ml}$  g) Cystone (500 $\mu\text{g}$  / ml)**

**DISSCUSION**

Several studies are carried out that the Microscopic images are to validate the results obtained by the aggregation method. The results of the % inhibition data are further confirmed by microscopic images of calcium oxalate crystals. The result of the microscopic image indicated that the inhibition of crystal is done in different concentration of EEGG extract [500 $\mu\text{g}$  / ml, 250 $\mu\text{g}$  / ml, 100 $\mu\text{g}$  / ml, 50 $\mu\text{g}$  / ml, and 10 $\mu\text{g}$  / ml], control and standard drug (Cystone 500 $\mu\text{g}$  / ml).

By comparing the control, different concentration of sample with standard drug. The size and quantity of the calcium oxalate crystals decreases when the EEGG concentration increases. The visibilities of crystals are more in lower concentration and lesser visibility of crystals in higher concentration and standard drug cystone. The microscopic studies and Percentage inhibition calculation supported the results and it indicates that the aerial parts of *Gomphrena globosa* (L.) possess anti urolithiatic activity.

### SUMMARY AND CONCLUSION

The best way to treat the urolithiasis is to control the process of crystallization in human body. Many natural plants and herbal extracts are widely used for urolithiasis from the ancient times. Mostly they are not scientifically proven. So this study is mainly conducted to identify the anti urolithiasis in the aerial parts of *Gomphrena globosa* L. [EEGG]

We concluding with the report of the Preliminary Phyto chemical tests, aggregation activity and by the microscopic images, the aerial parts of *Gomphrena globosa* L. [EEGG] has the anti urolithiatic activity.

Even though the plant extract of EEGG is less effective than the usual medication Cystone, both the percentage inhibition data and the microscopic findings support that it has a dose-dependent anti-urolithiatic activity. Although more research is needed to determine the plant extract's efficacy for clinical application, it may be a useful substitute for treating urolithiasis.

From the present study it is concluded that the ethanolic extract of *Gomphrena globosa* (L.) could dissolve the urinary stones without any surgical aid. It is the first hand report of Anti urolithiatic activity in *Gomphrena globosa* (L.). Further in vivo studies and clinical trials are required for the scientific research. The present investigation of in vitro research will be support as scientific documentation for the further research works.

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