

## Evaluation of Anti-Urolithiasis activity of *Eleusine coracana* seeds extract by in-vitro Study.

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**Abstract:** Urolithiasis is very common now a days. *Eleusine Coracana* is a millet, capable to show lots of pharmacological activities like antibacterial activity, anti-oxidant, anti-diabetic, Immunomodulatory activity. It is known as *Eleusine Coracana* and belongs to Poaceae family. It is cultivated in Garhwal region of Uttarakhand. It has consisted different chemical constituent like flavonoids, phenols, alkaloids, glycosides, phenolic compounds etc. Extraction of millet was done by Soxhlet apparatus. Anti-urolithiasis testing was done by the Aggregation and Nucleation assay method and check the effectiveness of plant extract by observing the dissolving of crystals of calcium oxalates in the stock solution of millet. It was observed that 40mg/ml was best for anti-urolithiasis activity of millet. By performing the phytochemical test, indicated the presence of glycosides, phenolic compounds, flavonoids, saponins, alkaloids etc. the absorbance of *Eleusine Coracana* was 620nm.

**Keywords:** *Eleusine coracana*, Soxhlet apparatus, Anti – Urolithiasis, Phytochemicals.

### INTRODUCTION

Urolithiasis is a Genitourinary tract condition in which stones exist in the renal pelvis and then start to move to urinary system like urinary bladder, urethra and ureters. The term urolithiasis refers to the formation of Calculi and stones in Kidney or Urinary system. The stones which are formed in Kidney or Urinary system, 80% made up of Calcium and Phosphate. Other stones are made up of Uric acid, Struvite and Cysteine<sup>1</sup>. The common risk factors for Urolithiasis are poor oral fluid intake, environmental factor, high animal protein intake, high oxalate consist vegetables intake (like beans, Chocolate, teas, nuts, soda, spinach) and high salt intake. Oral hydration helps to increase urine production or formation of 2.5L urine daily. Other than its Coffee, Water, low sugar fruits, grapes etc also helps in the production of urine. Some of the medical conditions can also cause Urolithiasis like hypertension, diabetes mellitus, obesity, malignancy, gout etc. uric acid stone formation can also occur due to low urinary uric acid levels, low pH, low urinary volume<sup>2</sup>.



Fig.(1) Seeds of *Eleusine coracana*



Fig.(2) Aerial part of *Eleusine coracana*

Lots of medication are available to treat urolithiasis which is called as Anti – Urolithiasis drugs. NSAIDs, Opioids and Diuretics use in the treatment of Urolithiasis<sup>3</sup>. As we already know that allopathic medicines produce pharmacological effect but they are also responsible to cause lots of side effects and can increase the chances of organ failures. In Uttarakhand both Garhwali and Kumauni's don't use allopathic medicines they use traditional herbs, plants to treat their diseases like herbs related to Echinacea family helps to improve body's immune system, Flaxseeds, Garlic are used to reduce Cholesterol level<sup>4,5</sup>, Feverfew helps to treat headache and also good for menstrual cramps. *Eleusine coracana* is also a medicinal herb which found in Garhwali region other than Garhwal it also found in Nepal and East African region. It grows in dry winter season so it is a rabi crop<sup>6,7</sup>. It belongs to Poaceae family and it is also known as *Eleusine Coracana*. Millets are group of cereal crops grown worldwide for food and staples in Harsh environment. The family of *Eleusine Coracana* is sub family is Panicoideae. The *Eleusine Coracana* is the best sources of the calcium and protein, which provide the phytochemical which can fight against diseases<sup>8,9</sup>.

## MATERIAL AND METHOD:

2.1 Collection and Authentication: *Eleusine coracana* is commonly found in Garhwal region of Uttarakhand state in India. I have collected the seeds of *Eleusine coracana* from the Tehri Garhwal region of Dehradun, Uttarakhand, India and get authentication from Saheed Durga Mall Govt. Post Graduate College, Doiwala, Dehradun by the Assistant Professor, Dr. Anil Kumar (Department of Botany). After the authentication, dry the seeds at room temperature and packed them into air tight container.

2.2 Selection of solvent: Solvent Ethanol contains the soluble phytochemical during extraction, which can help for anti-urolithiasis activity of *Eleusine Coracana*.

2.3 Extraction: For the extraction of *Eleusine coracana*, Soxhlet extraction method has used for extraction. Firstly, collect the millets, wash them and then dry. After drying the millets convert into coarse powder with the help of pestle mortar. Make a thimble and pack powder into the thimble by using Soxhlet extraction method<sup>11</sup>.

Take 50gm of coarse powder of millet and 500 ml of ethanol as solvent. Ration of drug: solvent is 1:10 and temperature required for extraction process is 50° C. this process has taken 40-45 hours. After completing the extraction process. Dry the extract by using water bath at 60-70° C. after drying the extract material, weight it. Weight of dried material is 1. 8gm. The percentage yield of *Eleusine coracana* seeds extract was 3.48 %



Fig.(3) Extraction of *Eleusine Coracana* millets

## 2.4 Phytochemical testing:

Table (1) Phytochemical testing

S.No	Test	Procedure	Results
	Alkaloids	Dragendroff test: - Few ml filtrate + 1 ml reagent	Redish brown colour
	Flavonoids	Lead acetate: 1 milliliter of plant extract plus a few drops of 10% lead acetate unite to form a yellow precipitate.	A yellow precipitate
	Phenols	Lead acetate solution: 1 milliliter of plant extract with 3 milliliters of 10% lead acetate	Bulky white precipitate
	Cardiac glycoside	Bromine water: - a little amount of bromine water and plant extract	Yellow precipitate
	Saponins	Froth test: mix extract with water and shake it.	Foam produce
	Terpenoids	Extract + Chloroform + Sulphuric acid	Reddish brown color produce.

2.5 Formation of Calcium oxalate crystals/stones: The stones or crystals which are found in Kidney and responsible to cause Urolithiasis. These stones are made up of Calcium oxalate and phosphates. For anti-urolithiasis activity, stones have formed artificially by adding some water into the mixture of calcium oxalate and phosphate.

2.6 Anti – Urolithiasis activity: Firstly, prepare stock solution of with concentration of 100mg/ml and then divide stock solution into test tubes (20,40,60,80,100mg). add formed crystals into the test tubes and wait for some time. Observe the test tubes and check the crystals has dissolved or not. For the comparative study of *Eleusine Coracana* for anti-urolithiasis activity, Cystone has used as a standard drug<sup>12,13</sup>.

2.7 UV visible spectroscopy: For evaluating the absorbance of *Eleusine Coracana*, use UV visible spectroscopy method.

### In-vitro Methods

3.1 Aggregation Assay: It is completely based on percentage of crystal dissolution. It shows that turbidity increase with increase in dissolution and measured at 620nm. The aggregation assay was employed to evaluate the ability of *Eleusine Coracana* seeds extract to inhibit the aggregation of calcium oxalate crystals. The *Eleusine Coracana* seed extract exhibited a dose dependent decrease in absorbance, demonstrating progressive inhibition of calcium oxalate crystal aggregation<sup>14</sup>.

3.2 Nucleation Assay: It inhibit crystallization by inhibiting nucleation of calcium oxalate through into disintegrating into smaller particles with increasing concentration of fraction. The inhibitory activity of the extracts on the nucleation of calcium oxalate crystals was determined by a spectrophotometric assay.

### Result

#### 4.1 Phytochemical testing:

Table (2) Result of Phytochemical testing of *Eleusine coracana*

S. No	Test	Procedure	Observations	Results
	Alkaloids	Dragendroff test: - Few ml filtrate + 1 ml reagent	Redish brown colour	Positive

	Flavonoids	Lead acetate: 1 milliliter of plant extract plus a few drops of 10% lead acetate unite to form a yellow precipitate.	A yellow precipitate	Positive
	Phenols	Leadacetate solution: 1 milliliter of plant extract with 3 milliliters of 10% lead acetate	Bulky white precipitate	Positive
	Cardiac glycoside	Brominewater: - a little amount of bromine water and plant extract	Yellow precipitate	Positive
	Saponins	Froth test: mix extract with water and shake it.	Foam produce	Positive
	Terpenoids	Extract + Chloroform + Sulphuric acid	Reddish brown color produce.	Positive



Fig (4) Phytochemical testing of millet extract

4.2 Anti – Urolithiasis activity: After following the procedure, it was observed that 40mg/ml dose is good for the anti-urolithiasis activity of *Eleusine Coracana* drug<sup>16,17</sup>.



Fig (5) Anti-Urolithiasis activity of millet

4.3 UV visible spectroscopy: Absorbance was measured at 620nm, where lower absorbance correlates with higher inhibition of crystals aggregation. The *Eleusine Coracana* seed extract exhibited a dose dependent decrease in absorbance, demonstrating progressive inhibition of calcium oxalate crystals aggregation. The result of *Eleusine Coracana* drug is following:

Table (3) Absorbance measured by aggregation Assay

Concentration (µg/ml)	Absorbance <i>E.coracana</i>	Absorbance Cystone
100 µg/ml	1.3	1.5
200 µg/ml	1.1	1.3
400 µg/ml	0.85	1.05
600 µg/ml	0.65	0.8
800 µg/ml	0.45	0.55
1000 µg/ml	0.25	0.35

Table (4) Absorbance measured by Nucleation Assay

Concentration (µg/ml)	Absorbance <i>E.coracana</i>	Absorbance Cystone
100 µg/ml	1.4	1.6
200 µg/ml	1.2	1.4
400 µg/ml	0.9	1.1
600 µg/ml	0.7	0.83
800 µg/ml	0.5	0.6
1000 µg/ml	0.3	0.4

## DISCUSSION

The present in-vitro study aimed to evaluate the anti-urolithiasis activity of *Eleusine Coracana* seed extract. Urolithiasis, characterized by the formation of calculi (stones) in the urinary tract, remains a prevalent and recurrent condition with limited preventive pharmacological options. Our findings support the potential of *Eleusine Coracana* as a natural anti-urolithiasis agent, aligning with ethnomedicinal claims and recent phytopharmacological interests.

The extract of *Eleusine Coracana* seeds demonstrated significant inhibitory effects on calcium oxalate crystal nucleation, growth, and aggregation, the three critical stages in stone formation. These in-vitro results suggest that the bioactive compounds in the seed extract may interfere with crystal formation or promote disintegration of early stone structures. The efficacy observed may be attributed to the presence of flavonoids, polyphenols, saponins, and other phytochemicals known for their antioxidant and chelating properties. Antioxidants help reduce oxidative stress in renal tissues, which is a known contributor to stone formation. Additionally, the inhibition of calcium oxalate crystallization in this study is consistent with other plant-based anti-urolithic agents. Compared to the control, the extract significantly decreased the size and number of calcium oxalate crystals, indicating its potential to not only prevent stone formation but possibly aid in the breakdown of existing stones. The results were dose-dependent, further supporting a pharmacological mechanism of action. Despite the promising findings, several limitations must be acknowledged. The in-vitro nature of the study.

For the testing of anti-urolithiasis activity of *Eleusine Coracana*, Ethanolic extraction was used. For evaluating anti-urolithiasis activity of *Eleusine coracana*, 40mg/ml dose was required. The phytochemical testing shows the presence of glycosides, flavonoids, phenolic compounds, alkaloids, etc. UV visible spectroscopy shows that 620 nm absorbance of *Eleusine Coracana*.

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