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# Anti-Urolithiatic Activity of Cassia Auriculata Ethanolic Seed Extract in Wistar Rats

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***Abstract***

***Background:*** *Urolithiasis is a medical condition that, despite substantial research in the field of urology, has yet to find a cure within the allopathic medical approach. The process of stone development, known as nephrolithiasis, can occur within the kidney or any segment of the urinary tract, encompassing the ureters and bladder.* ***Material and Methods:*** *The seeds of Cassia auriculata (Linn.) were obtained in June 2021 from Mettukadai hamlet, located in the Erode District of Tamilnadu, India. The herbarium of the plant was meticulously assembled, verified, and afterwards deposited as a voucher specimen. The voucher specimen was retained within the college premises for future reference.* ***Results:*** *The current investigation involved conducting a preliminary phytochemical analysis on the ethanolic seed extract of Cassia auriculata Linn. The research revealed the existence of many phytochemical constituents, including Alkaloids, Flavanoids, Carbohydrate, Sterols, Phytosterols, Phenols, Terpenoids, Amino acids, and Anthraquinones. The acute toxicity experiments revealed that rats exhibited tolerance to a maximum dose of 2000 mg/kg body weight, and no discernible alterations in behavior were detected across all experimental groups. Hence, fractions equivalent to one-fourth and one-eighth of the maximum tolerated dose of 200 mg/kg body weight were selected for subsequent investigations.* ***Conclusion:*** *In summary, the findings suggest that the application of an ethanolic seed extract derived from Cassia auriculata Linn effectively decreased the progression of urinary stone formation. Additionally, it appears that the efficacy of the treatment impact surpasses that of its preventative counterpart.*

**Keywords:** *Cassia auriculata, Seed, Urolithiasis, Wistar rats*

## 1. Introduction

Urolithiasis is a disease that, despite substantial research in the field of urology, has shown to be incurable in allopathic medicine. The process of stone development, known as urolithiasis, can manifest in various locations within the urinary system, such as the kidney, ureters, and bladder [1, 2].

Urolithiasis exerts a significant impact on the healthcare system, exhibiting a prevalence above 10% and an anticipated recurrence rate of over 50%. The global prevalence of urolithiasis is considerable, with calcium oxalate stones accounting for about 80% of urinary calculi, either as pure calcium oxalate stones or in combination with calcium phosphate [3]. Epidemiological investigations have demonstrated a higher prevalence of nephrolithiasis in males compared to females. Additionally, this condition is found to be more widespread in individuals between the ages of 20 and 40, regardless of gender. The occurrence of stone formation is influenced by various circumstances, including geographical, climatic, ethnic, nutritional, and genetic factors [4-6].

The utilization of intravenous urography enables the acquisition of pertinent data pertaining to renal functionality, the structural composition of the collecting system, and the identification of potential obstructions. Non-contrast-enhanced computed tomography (CT) enables swift capture of three-dimensional data, providing valuable insights into stone dimensions, density, skin-to-stone distance, and adjacent anatomical structures [7, 8]. However, this imaging technique is associated with the drawback of heightened radiation exposure. The efficacy of low-dose and ultra-low-dose protocols appears to be similar to that of standard-dose protocols, except for the identification of extremely small stones or stones in individuals with obesity. The utilization of invasive interventions in the management of nephrolithiasis can lead to significant consequences and impose a substantial financial burden on the healthcare system. On the other hand, traditional medicines have served as an alternative treatment for certain ailments and have also contributed supplemental insights into the pathophysiology of diseases [9,-11].

The etiology of calcium oxalate renal calculi formation has garnered significant interest among medical researchers due to its prevalent clinical manifestation and the challenges associated with its management. Hyperoxaluria is considered to be a significant contributing factor to the development of idiopathic calcium oxalate disease in humans [12, 13]. The primary component responsible for stone formation, oxalate, has been observed to trigger lipid peroxidation, resulting in the breakdown of cellular membrane integrity. Lipid peroxidation refers to the oxidative degradation of polyunsaturated lipids, which is initiated by the presence of free radicals. This phenomenon induces changes in the fluidity and permeability of the cellular membrane, consequently impacting the transportation of ions across the organelle [14-16].

Cassia auriculata Linn., belonging to the family Leguminosae, is commonly referred to as Avaram tree. The arrangement of the leaves is alternate, with stipules, and they are paripinnate compound. The leaves are abundant and closely spaced, with a rachis of 8.8-12.5 cm in length. The rachis is narrow, wrinkled, slender, and covered with fine hairs. Additionally, there is an upright linear gland present between each pair of leaflets. The leaflets themselves number between 16 and 24, and they have very short stalks. The dimensions of the object are approximately 2-2.5 cm in length and 1-1.3 cm in width. The shape is slightly overlapping, with an oval oblong appearance and obtuse ends. The surface is either smooth or minutely covered in fine hairs, and the color is a dull green, with a paler shade on the underside. The stipules, which are big and reniform-round in shape, are located at the base and extend into a filiform point on the side of the adjacent petiole, remaining persistent [17-19].

The recommended approach involves pulverizing the substance into a fine powder and gently administering it through a controlled airflow into the eyes that are experiencing the condition. Seeds has several medicinal properties such as astringent, sour, cooling, constipating, depurative, aphrodisiac, anthelmintic, stomachic, and alexeteric. They have been found to be beneficial in managing conditions such as diabetes, chyluria, ocular disorders, dysentery, diarrhea, swellings, abdominal disorders, leprosy, skin illnesses, worm infestations, and chronic purulent conjunctivitis [20, 21].

The objective of this study is to evaluate the potential protective properties of the ethanolic seed extract of Cassia auriculata Linn. against urolithiasis produced by ethylene glycol. This will be achieved by conducting pharmacological and biochemical investigations using the aforementioned extract.

## 2. Materials and Methods

### Collection and authentication of plant

The seeds of Cassia auriculata (Linn.) were obtained in June 2021 from Mettukadai hamlet, located in the Erode District of Tamilnadu, India. The herbarium of the plant was meticulously assembled, verified, and afterwards deposited as a voucher specimen. The voucher specimen was retained within the college premises for future reference.

**Sample Preparation**

The seeds obtained from the collected plants were subsequently isolated and subjected to a drying process at ambient room temperature. The desiccated seeds of the botanical specimen were carefully preserved within hermetically sealed receptacles till the commencement of the analytical investigation.

**Preparation of extract**

A quantity of 25 grams of pulverized seeds is combined with 250 ml of ethanol in a distinct beaker. This mixture is afterwards introduced into a round bottom flask, which is then connected to a Soxhlet extractor and condenser apparatus placed on an isomantle. The pulverized botanical matter is carefully inserted into the small cylindrical container known as the thimble, which is afterwards positioned into the apparatus known as the Soxhlet extractor. The side arm is insulated with glass wool. The solvent is subjected to heat using the isomantle, resulting in its evaporation. The powder was subjected to solvent extraction using a volume of 250 ml at a temperature of 40˚C for a duration of 24 hours. The solution containing bioactive compounds was transferred to a rotary evaporator in order to remove the solvent and get a solid extract. The specimen was stored in a cryogenic environment at a temperature of -4˚C in order to facilitate its utilization in subsequent research endeavors.

***In-Vitro* Antioxidant Activity**

**Radical Scavenging Activity of DPPH**

Hatano et al.'s approach was used to determine the extent to which an item may scavenge DPPH. Here, both the extract solution and the standard were removed from their respective 1ml bottles. After adding 5 ml of DPPH solution in methanol and shaking the mixture thoroughly, the solutions were incubated at 370C for 20 minutes. At 517 nm, the absorbance was measured against a blank of methanol. As a standard, DPPH absorbance was measured. The experiments were repeated three times for accuracy. The formula for determining the percentage of anti-radical action is as follows:

$$\%\ \text{Antiradical activity} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\textit{Control Absorbance}} \text{X } 100$$

**Total Phenolic Content**

Using the Folin-Ciocalteu technique, we shall determine the total phenolic content. TOA 25 ml volumetric flask holding 9 ml of doubly distilled water will be prepared by adding 1 ml of approximately diluted samples and a standard solution of gallic acid. A blank reagent will be made with double-distilled water. The sample solution (0.5 ml), Folin-ciocalteu phenolic reagent (2.5 ml), and 7.5% Na2CO3 solution (2.0 ml) will be combined and stirred. After 30 minutes of incubation in the dark at room temperature, the absorbance of the reaction mixture was measured at 760 nm.

**Total flavonoid content**

Ordonez et al.'s technique is used to calculate the total flavonoid content of a sample. After incubating the sample (1mg/ml) in 2 ml of 2% AlCl3 ethanol solution at room temperature for 1 hour, the absorbance at 420 nm was measured using a Perkin-Elmer UV/V is lambda spectrophotometer. Flavonoids are easily identifiable by their characteristic yellow hue. Using the calibration curve equation, total flavonoid content is expressed as quercetin equivalent (mg/g).

**Pharmacological Studies**

**Acute Toxicity Studies**

The OECD guidelines were used in the acute toxicity studies. There are three female animals used at each stage of the process. Acute toxicity can be determined in an average of two to three steps, though this can vary widely depending on the mortality and/or morbidity of the test animals. It reduces the

number of animals used while yet providing sufficient information for valid scientific conclusions. Test substances can be ranked and classified using the Globally Harmonized System for the categorization of compounds that induce acute toxicity thanks to the procedure's usage of defined doses.

## 3. Results and Discussion

### Preparation of plant extracts:

The Soxhlet apparatus was utilized to extract the seeds of Cassia auriculata Linn., weighing 1000 g, which had been previously dried. The extraction process was carried out for a duration of 24 hours. The quantity of the Cassia auriculata Linn. Extract obtained was 320.4 grams. The experiment yielded a percentage of 32.4%.

### Initial phytochemical investigation of cassia auriculata Linn. Seed extract:

Table 1 displays the findings obtained from the initial phytochemical investigation conducted on the ethanolic seed extract of Cassia auriculata Linn.

**Table 1:** Phytochemical Constituents of Cassia auriculata Linn

| Sr. No. | Constituents | Ethanolic Extract |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | + |
| 3 | Carbohydrates | + |
| 4 | Glycosides | _ |
| 5 | Saponins | _ |
| 6 | Tannins | + |
| 7 | Phytosterols | + |
| 8 | Proteins | _ |
| 9 | Aminoacids | + |
| 10 | Anthraquinones | + |
| 11 | Phenols | + |

**+ Present; - Absent**

### Acute Oral Toxicity Study

The investigation on acute oral toxicity was conducted in accordance with the requirements outlined by the Organisation for Economic Co-operation and Development (OECD) 423. Three male rats were orally administered a beginning dose of 2000 mg/kg body weight of an ethanolic seed extract of Cassia auriculata Linn. The rats were thereafter examined. No instances of fatality, mortality, or hazardous responses were seen at any of the indicated dose levels during the duration of the study. Table 2 presents the findings of acute oral toxicity investigations.

**Table 2:** Cassia auriculata Linn ethanolic seed extract total phenolic content

| Sample | TPC(mg GAE/µL extract) |
|---|---|
| Extract | 88.30 ± 2.30* |

**Table 3: Cassia auriculata Linn ethanolic seed extract total flavonoids**

| Sample | TFC(mg QE/µL extract) |
|---|---|
| Extract | 50.473 ± 0.657** |

**Table 4:** Animal groups details

| Groups | Drugs / extracts | Treatment Protocol | Animals | Route of Administration | Dose |
|---|---|---|---|---|---|
| 1. | Control | 28 days | 6 | Oral | Ad libitum |

| | | | | | |
|---|---|---|---|---|---|
| 2. | Ethylene glycol in water | 28 days | 6 | Oral | 0.75% v/v |
| 3. | Ethylene glycol (0.75% v/v) in water + 750 mg/kg cystone | 28 days | 6 | Oral | 750 mg/kg b.w |
| 4. | Ethylene glycol (0.75% v/v) in water + extract-I | 28 days | 6 | Oral | 250 mg/kg b.w |
| 5. | Ethylene glycol (0.75% v/v) in water + extract-II | 28 days | 6 | Oral | 500 mg/kg b.w |

The study's primary objective is to create a novel herbal treatment for urolithiasis. Despite their widespread availability, many of the medications now used to treat the disorder's symptoms are fraught with undesirable consequences. In addition, the fundamental cause of the disease remains untreated while the medications treat only the symptoms. Dizziness, weakness, increased hallucinations, nausea, vomiting, muscle cramps, lack of appetite, insomnia, disrupted sleep, diarrhea, nephritic diseases, etc. are all side effects caused by synthetic drugs [22, 23].

Herbal medications, on the other hand, have fewer negative effects and are affordable for all socioeconomic groups. Because of this, uroprotective medicines derived from natural sources, such as herbs, are needed to replace their synthetic counterparts. Traditional medicine and folklore both attest to the effectiveness of plant extracts as anti-urolithiatic agents. The protective effect of the ethanolic seed extract of Cassia auriculata Linn was assessed using the same mode of administration as is customary for traditional medicines. Urolithiasis was generated in rats by ethylene glycol. Urinary stone disease treatment has benefited greatly from the findings of the therapeutic roles of various herbal medicines as an alternative or supplemental therapy [24, 25].

Most likely, kidney stones are the result of a combination of a person's genetic predisposition and environmental (i.e., diet and lifestyle) triggers. Since the urinary system of male rats is most similar to that of humans, and because previous research has indicated that the amount of stone deposition in female rats is much smaller, male rats were chosen to induce Urolithiasis in the present investigation. Cassia auriculata Linn. seeds were extracted using ethanol, and a preliminary phytochemical examination revealed the presence of Alkaloids, Flavanoids, Carbohydrate, Sterols, Phytosterols, Phenols, Terpenoids, Amino Acids, and Anthraquinones. In acute toxicity investigations, no significant behavioral abnormalities were seen at the highest dose of 2000 mg/kg body weight. Accordingly, we decided to conduct our experiments using 14 and 18 of the 200 mg/kg b.w. maximum tolerated dose [26, 27].

The ethanolic seed extract of Cassia auriculata Linn. showed antioxidant activity that was on par with the standard at all tested doses in invitro antioxidant experiments. Antioxidant activity, as measured by DPPH, reducing power, and nitric oxide, increased linearly with concentration. Antioxidants and free radical scavengers, polyphenolic substances have long been recognized for their ability to protect cells from oxidative damage. Researchers found that flavonoids and phenols were present in the extract as well. Because of these chemicals, the extract may be more widely used locally for the treatment of illnesses brought on by oxidative stress. Calcium, magnesium, and phosphate were excreted in greater amounts after prolonged injection of 0.75 percent ethylene glycol, which caused hyperoxaluria. Significant reductions in calcium, magnesium, and phosphate were observed after Cassia auriculata Linn. ethanolic seed extract was administered. Calcium, phosphate, and magnesium crystals are more likely to accumulate in the kidneys of stone-forming rats [28, 29].

Both regimens' renal concentration of stone producing elements were dramatically reduced after treatment with an ethanolic seed extract of Cassia auriculata Linn. blood creatinine was elevated in Group II, indicating severe renal impairment, whereas blood urea, uric acid, and BUN were all considerably higher in calculi-induced animals compared to controls. However, blood creatinine, urea, uric acid, and BUN levels were dramatically reduced by the ethanolic seed extract of Cassia auriculata Linn. Multiple in vitro and in vivo investigations have suggested that exposure to high concentrations

of oxalate can have deleterious effects on renal architecture via intracellular oxidative stress, subsequent alterations in membrane structure, membrane lipid peroxidation, and ultimately cell death. In the current investigation, it was found that ethylene glycol treatment raised kidney MDA content and lowered antioxidant enzyme activity. The effects of oxidative stress were mitigated by the ESCA - I & II treatment. Crystal formation has been demonstrated in multiple studies to harm cells, cause them to detach from the basement membrane, and produce degradation chemicals that further encourage crystal nucleation. Because epithelial damage reveals a range of crystal adhesion molecules on epithelial surfaces, it facilitates crystal retention in the kidneys [30-32].

## 4. Conclusion

Urolithiasis is characterized by a decrease in glomerular filtration rate as waste products like urea and uric acid collect in the blood as a result of a blockage to the outflow of urine caused by stones in the urinary system. Significant kidney damage has occurred. The data show that treating patients with an ethanolic seed extract of Cassia auriculata Linn. considerably slowed the development of urinary stones. The therapeutic benefit appears to outweigh the preventative one. Possible mechanisms include its diuretic impact, antioxidant capacity, nephroprotective nature, and the ability to reduce the concentration of urinary stone-forming components. Additional laboratory and clinical research is needed to determine the chemical components of the extract and the mechanism responsible for its pharmacological actions.

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None

**Conflict of Interest**

None

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