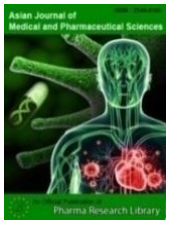

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In-Vitro anti-Urolithiatic activity of *Quisqualis indica*– Flower oil

B. Siva Prasad[1], K. Siva Prasad[1], T. Vijaya*[.1], K Gangi Naidu[1], KL Shireesha Rao[1]
[1]Department of Botany, S V University, Tirupati, A.P, India.

A B S T R A C T
The present study was undertaken to evaluate the *in-vitro* antiurolithiatic activity of the medicinal plant flower *Quiscalis indica*. Oil extract showed its maximum efficiency in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the ethanolic flower extracts of *Quiscalis indica* were quite promising for further studies in this regard. In this study cystone was used as standard drug (22%unique).
Keywords: *In-vitro* anti-urolithiatic activity, ethanolic extract, urolithiasis, *Quiscalis indica*, cystone.

A R T I C L E I N F O

***Corresponding Author:**
T. Vijaya
Department of Botany,
S V University,
Tirupati, A.P, India.

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C o n t e n t s

1. Introduction

Plants constitute a fundamental component of life on Earth, playing a crucial role in the well-being of both animals and humans. They not only serve as a source of substance but also contribute raw materials for medicine and fulfill various essential needs that sustain life, dating back to the origins of humanity. In contemporary medicine, numerous plant-derived compounds are integral as therapeutic agents, highlighting the significance of plants in healthcare. The excessive use of synthetic drugs has led to a rise in adverse drug reactions, prompting a shift towards natural remedies. Herbs and herbal drugs have garnered attention due to their clinically proven effects, creating a growing interest among the populace. Consequently, there is an urgent requirement for thorough scientific validation of traditional medicinal plant drugs to ascertain their efficacy and safety within the framework of modern science. Over recent generations, the knowledge surrounding medicinal plants and their practical applications has gradually diminished, yet there's now a notable resurgence. Plants serve multifaceted roles crucial for sustaining daily life. They act as providers of substance in various ways: serving as a primary source of nourishment for humans and animals, generating their own food through photosynthesis while also being utilized by other organisms for their nutritional needs. Moreover, they play a vitol role in regulating the water cycle, facilitating the movement of water from the soil to the atmosphere, a process known as transportation. Additionally, through the absorption of carbon dioxide and the subsequent release of oxygen into the air, plants supply the essential element vital for the respiration of all living beings. Furthermore, numerous plants serve diverse medicinal purposes, underscoring their significance in healthcare and therapeutic treatments.

Kidney stones manifest as a multi-factorial disorder, arising from the combined influence of epidemiological, biochemical, and genetic risk factors. These rigid formations result from the amalgamation of minerals and acid salts in concentrated urine, potentially causing discomfort during their passage through the urinary tract,

although typically without causing permanent damage. Urolithiasis, the presence of solid non-metallic minerals in the urinary system, ranks as the third most prevalent ailment affecting the urinary tract. It involves a complex process characterized by an imbalance between promoters and inhibitors within the kidney. The formation of kidney stones encompasses various phytochemical events, commencing with crystal nucleation, aggregation, and concluding with retention within the urinary system. Among the diverse types of kidney stones, calcium oxalate stones are the most prevalent, constituting up to 80% of analyzed stones. These calcium-containing stones manifest in the form of pure calcium oxalate (50%), calcium phosphate (5%), and a combination of both (45%), followed by magnesium phosphate (15-20%), uric acid (10%), and cystine (1%). In the developed world, it is approximately stated that a minimum of 10% of the population is affected by urinary tract diseases, and within this category, kidney stones are prevalent, with an annual incidence ranging from 0.5% to 1.9%. In the context of India, around 12% of the population is anticipated to experience urinary stones, and within this group, 50% of cases involve the loss of one or both kidneys, accompanied by varying degrees of renal damage, with or without associated complications.

The prevalence of stone disease is notably higher in males, being 2-3 times more common than in females. The recurrence rate is substantial, with males experiencing a recurrence rate of 70-81% and females facing a rate of 47-60%. Despite significant advancements in understanding the pathophysiology and treatment of urolithiasis, there is currently no satisfactory pharmaceutical intervention employed in clinical therapy. Existing procedures such as kidney dialysis, endoscopic stone abstraction, and extracorporeal shock wave lithotripsy are hindered by their prohibitive costs, and recurrence remains a prevalent challenge associated with these methods.

Information gathered from both in vitro and in vivo clinical trials suggests that phototherapeutic agents hold promise as an alternative approach to managing urolithiasis. The efficacy of medicinal plants and their derivatives is particularly notable, as they actively support natural repair mechanisms. Exploring the pharmacological and phytochemical aspects of medicinal plants, rooted in traditional knowledge, can unveil new drugs and contribute to the development of pharmacologically significant products for human healthcare. Green medicines, derived from plants, are considered safer and more reliable compared to expensive synthetic drugs, many of which carry potential side effects. This underscores the potential of plant-based alternatives as a safer and dependable option in the realm of healthcare.

2. Materials and Methods

Collection of plant material

Fresh flowers of *Quisqualis indica* were collected from botanical garden Dept. of Botany, S V University, Tirupati. The flowers were identified and authenticated by Prof. N. Savithramma, Professor, Dept. of Botany, S V University, Tirupati. Voucher specimen is deposited in the Herbarium of Botany department Sri Venkateswara University.

In-vitro Anti-Urolithiatic Assay (Calcium oxalate dissolution assay)

Preparation of Plant Extract

Fresh flowers of *Quisquails* were collected, washed with alcohol and subjected to maceration using olive oil for 30days. Further it was extracted using filter paper and subjected phytochemical screening followed by GC-MS analysis

Preparation of Semi Permeable Membrane

The apex of the egg was punctured to remove the entire content from it. The empty eggs are washed with distilled water and placed in a beaker containing 8ml Con. HCl in 400ml distilled water. This was kept for an overnight. It results in the complete decalcification of the semipermeable egg membrane. On the next day, the semipermeable membranes were removed from the eggshells carefully. The obtained semipermeable membranes are washed properly with distilled water and are placed in the ammonia solution. Later, rinse again with the distilled water and keep in the refrigerator at a p^H 7-7.4 in the moistened condition until the time of use.

Preparation of Positive Control

0.5g of Cystone® tablet was placed in absolute ethanol for about an hour for removing the colour coating. The tablet was then crushed into a powder form and dispersed in 100 ml distilled water and filtered out. The filtrate obtained was used as the positive control for the in-vitro anti-urolithiatic assay.

Chemicals

Sodium oxalate, Calcium chloride dehydrate, 0.1M Trisbuffer, con. HCl, 1N H_2SO_4, Pottassium permanganate, Ammonium solution.

Synthesis of Calcium Oxalate by Homogenous Precipitation

In a beaker containing 100ml distilled water, dissolve 1.47g of calcium chloride dehydrate and, in another beaker, containing 100ml 2N H_2SO_4, dissolve 1.34g sodium oxalate. Both the contents were mixed in a beaker and stir constantly. The constant stirring precipitates out calcium oxalate. The formed calcium oxalate was washed with ammonium solution to remove the traces of H_2SO_4 and then wash with distilled water. Then it was allowed to dry at a temperature of 60^0C in an oven for 5 hours.

Preparation of 0.02M $KMnO_4$ Solution

In a beaker containing 100ml of distilled water, dissolve 0.32g $KMnO_4$ and boil it for 30 minutes. After cooling, the excess of MnO_4 was removed by the filtration process.

Estimation of Calcium Oxalate Dissolution:

Blank: 1mg calcium oxalate+1ml distilled water

Control: 1mg calcium oxalate + 1ml standard drug (Cystone®)

Group 1: 1mg calcium oxalate + 1 ml extract in distilled water

Group 2: 1mg calcium oxalate + 1 ml extract in ethanol

Group 3: 1mg calcium oxalate + 1 ml extract in chloroform

Group 4:1mg calcium oxalate+1ml extract in ethylacetate

Group 5: 1mg calcium oxalate + 1 ml extract in lime juice

Group 6: 1mg calcium oxalate +1ml extract in tender coconut water

Each of the six groups, blank and control were packed separately in eight different semipermeable membrane. The open end of the membrane was tied carefully with a thread and was suspended in conical flasks, each containing 100 ml 0.1 M Tris buffer. The end of the thread was tied on a stick placed on the mouth of the conical flask and was covered with aluminum foil. The whole set up was then kept in an incubator, preheated to 37^0C for 4 hour and kept for 3 days. After 3 days of incubation, the entire content in each membrane was removed by gently piercing the semipermeable membrane and was transferred in to four individual test tubes carefully. To each of the test tube, 4 ml of 1 N H_2SO_4 and 0.06- 0.08 ml of 0.02 M KMnO4 were added and kept aside for 2 hours. The colour change was observed and recorded. The change in the intensity of the colour was measured spectrophotometrically at 620 nm.

$$\% \text{ dissolution} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

3. Results and Discussion

The study evaluates the anti-urolithiatic activity of oil extract has been tested as compared with the standard cystone tablets and out of the six extracts, oil extract has the greater capability to dissolve calciumoxalate, which is the foremost element of kidney stone. It shows the highest percentage of dissolution (95.14%) equals to that of standard drug.

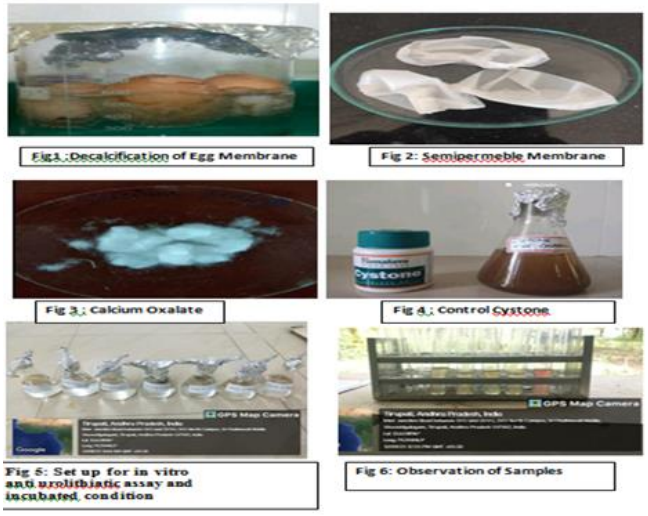
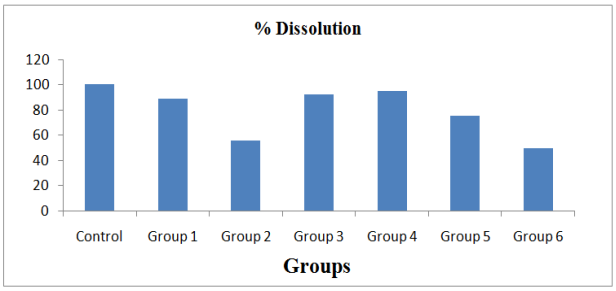


Table 1: Spectrophotometric Measurement and Percentage Dissolution of Calcium Oxalate.

Groups	% Dissolution
Control	100
Group1	88.83
Group2	55.82
Group3	91.99
Group4	95.14
Group5	75
Group6	49.75



Discussion:

Phytotherapy uses herbal drugs that are reported to be effective in the treatment of Urolithaisis with least side effects. Herbs also have the efficacy to improve the renal functions and to regulate the oxalate metabolism which helps to reduce there occurrence of renal calculi. In Ayurvedic system of medicines *Quisqualis indica* have a vital position in the treatment of body pains, piles, and toothache. Its extract is utilized to cure swellings, rheumatic infections, stiffness of joint sandurinary infections (Aron *et al*, 2013). *Quisqualis indica* was reported for its anti-diabetic, anti-inflammatory, anti-arthritic, wound healing, analgesicandanti-microbialactivitiy (Neyanila *et al*, 2013). Phytochemically the plant has been reported to contain flavanoids, quinone, tannins, phenolic acids, saponins, vaniliic acid and syrigic acid (Nair G.G.,1986). The presence of phytochemicals are considered as the responsible factor for the anti urolithiatic potential of the plant, which can be confirmed by the in-vivo analysis.

4. Conclusion

From the present study, it has been concluded that the extracts of *Quisqualis indica* produced significant in-vitro anti-urolithiatic activity in the dissolution of calcium oxalate when performed in presence of standard drug, Cystone®. Concluding that the extracts of *Quisqualis indica* are beneficial in the treatment of kidney stones. This focuses into the field of phytotherapy which uses herbal drugs for safer treatment of urolithiasis. This study has given the primary evidence for *Quisqualis indica.* The plant which possesses anti-urolithiatic property. However, to develop a potent anti- urolithiatic agent from this plant, the in-vitro results should be confirmed by the in-vivo analysis.

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