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Original Research Article

In vitro Pharmacological Evaluation of Herbal Tea for the Treatment of Urolithiasis

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ABSTRACT

Urolithiasis arises from the precipitation and crystallization of stone-forming minerals in the kidneys due to urine supersaturation. Calcium oxalate are the most prevalent among the stone-forming minerals. In Ayurveda, herbal remedies have been used in the treatment of urolithiasis. This study aimed to evaluate herbal tea extracts of *Trigonella foenum-graecum L.* leaves, *Mimosa pudica L.* leaves, and *Zea mays L.* silk for their anti-urolithiatic activity *in vitro*. Three formulations of herbal tea bag of the combination of *Trigonella foenum-graecum* Leaves, *Mimosa pudica* Leaves, and *Zea mays* silk were prepared in the following proportions; 50:25:25 (formulation 1), 25:50:25 (formulation 2), and 25:25:50 (formulation 3). Calcium oxalate crystals were precipitated by reaction between calcium chloride and sodium oxalate. The anti-urolithiatic effect of the herbal tea formulations was investigated via the nucleation and aggregation assays. The drug cystone was used as the standard. The calcium oxalate crystals in both assays were examined microscopically to determine their sizes, shapes and quantities. Results showed that the three herbal tea formulations exhibited significant ($p < 0.05$) inhibition of calcium oxalate crystal both in the nucleation and aggregation assays. The highest anti-urolithiatic effect was observed at 1.0 mg/mL for tea bag formulation 1, with percentage inhibition of 94.89%, and 81.45% in the nucleation and aggregation assays, respectively. Microscopic examination revealed a reduction of calcium oxalate crystals in the test samples compared to the control. This study has demonstrated the efficacy of herbal tea in dissolving calcium oxalate crystals and reducing stone formation, indicating its promising anti-urolithiasis activity.

Keywords: Urolithiasis, Herbal remedies, Calcium oxalate crystals, Anti-urolithiasis.

Introduction

The deposition of kidney stones in the urinary tract, a condition known as 'Urolithiasis', has become a popular urological problem in recent years. This medical condition is characterised by growth and deposits of solid-state deposits within the kidneys and through the urinary tract. Kidney stones can develop in various parts of the urinary system, primarily in the kidneys and ureters, and are likely less prevalent in the bladder and urethra. Urolithiasis is common in developed and developing countries, affecting millions of individuals worldwide, and can be extremely painful and uncomfortable. Patients with kidney stones can present with a wide range of symptoms. The symptoms of urolithiasis can range from mild to severe. Urolithiasis can present clinically with a sudden or gradual onset, typically manifesting as unilateral colicky abdominal or flank pain that fluctuates in intensity. Haematuria, or the presence of blood in urine, is a prevalent sign, with nearly 90% of cases revealing microscopic blood on urinalysis. Symptoms of nausea, vomiting and fever are often reported.[1,2]

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The condition is usually diagnosed using high-tech imaging examination technology, such as computed tomography or ultrasonography. In recent decades, Asian countries have seen an increase in urolithiasis cases. Researchers have found that this incidence varies greatly across the continent, ranging from 5% to over 19.1%. Some factors that contribute to an increased prevalence of urolithiasis are age, lifestyle, and genetic. Southeast Asian countries such as Malaysia show a remarkable number of urolithiasis cases. This increased number is attributed to high temperatures and overexposure to sunlight. Moreover, the incidence of urolithiasis in Malaysia has also increased to 442.7/100 000 over the past few decades. One of the surveys conducted in three primary healthcare clinics in Sarawak reported the prevalence rate as 4.04% with a male to female ratio of 1.2 to 1%. It was found out that this ratio equally affected both males and females, with a higher incidence in the age group of 25 to 64 years. In another study, conducted among patients at University Hospital Sains Malaysia for a duration of five years (2012-2016), the prevalence of urolithiasis was reported as 1.8% with a male to female ratio of 1.35 to 1%. From this reports, the Malay ethnicity, which makes up 91.1% of all cases, accounted for a vast majority of patients diagnosed with urolithiasis.[5] However, urolithiasis recurrence rates do not differ significantly among Asian countries, ranging from 6% to 17% after one year, 21% to 53% after three to five years, and 60% to 80% as a lifetime risk. However, studies show that males experience a higher recurrence rate than females.[3]

Despite the identification of multiple kidney stone types, calcium oxalate and calcium phosphate are the most common, accounting for 80% of cases. Other less common types include uric acid (9%), struvite (10%), and cystine (1%) (Figure 1). Calcium is the common element found in all kidney stones.[6] Stones with a high composition of calcium are known as calcium oxalate stones, which sometimes are present in combination with phosphate (apatite or brushite) in majority (80%) of

cases.[1,6] Such stones form easily when foods containing high concentrations of calcium are consumed. While calcium phosphate deposits are comparatively less common from oxalate stones, metabolic disorders or conditions that raise urine pH are known to be linked to the latter condition. Factors such as primary hyperoxaluria are known to promote calcium oxalate deposition and encourage calcium oxalate stone formation. Moreover, issues like hyperparathyroidism and renal tubular acidosis are associated with the development of calcium phosphate stones. Similarly, patients whose diet are high oxalate can experience frequent gastrointestinal disorders.[6,7]

Herbal remedies derived from various plant parts, such as leaves, bark, roots, and seeds have been widely used for various health conditions. One such application is the use of herbal medicine as an antiurolithiatic agent, particularly in the prevention and management of urolithiasis. While conventional therapies exist for urolithiasis and are costly and often inaccessible, the problem of urolithiasis recurrence and the side effects associated with pharmaceutical drugs remain a challenge.[8,9] The history of stone development, often accompanied by inflammation and chronic pain, has led to a growing interest in herbal formulations aimed at preventing lithiasis-related suffering.[9] Polyherbal formulations are an integral part of Ayurvedic medicine, one of the oldest traditional medicinal systems. This approach involves combining multiple medicinal herbs to achieve enhanced therapeutic effectiveness, a concept based in polypharmacy and polyherbalism. The benefits of polyherbal formulations include achieving better therapeutic effects with lower doses, reducing the risk of side effects, and enhancing patient compliance. It is important to note that clinical trials are needed to ascertain the efficacy of herbal medicine in both polyherbal formations and other formulations.[10]

The worldwide occurrence of urolithiasis underscores the need for more potent and secure antiurolithiatic therapies. It highlights the adverse effects of current antiurolithiatic drugs, prompting researchers to explore natural compounds as potential remedies. This study consisted of a review of 457 plants belonging to 108 families available in various parts of the world, all of which are known for their antiurolithiatic properties. Based on the information gathered, three herbs *Trigonella foenum-graecum*, *Mimosa pudica* and *Zea mays* were selected, and used to formulate a polyherbal tea, which was then evaluated for anti-urolithiatic activity.

Trigonella foenum-graecum L. (Fenugreek) has been used for the treatment of kidney problems and kidney stones due to its ability to eliminate calcium oxalate stones in traditional Chinese herbal medicine.[11] Furthermore, researchers have recognized the potential of fresh fenugreek leaves to improve digestion, alleviate flatulence symptoms, and offer therapeutic benefits to individuals with a sluggish liver. Fenugreek has been reported as a source of the steroid diosgenin and has shown several pharmacological activities including antiurolithiatic, anti-inflammatory, antispasmodic, analgesic, immunological, antidiabetic, hypolipidemic, antibacterial, anthelmintic, and antioxidant properties.[12]

Mimosa pudica L. (sensitive plant) is a widely used plant with various medicinal benefits. Mexicans use it to treat conditions like depression, rheumatoid arthritis, skin wounds, premenstrual syndrome, anxiety, menorrhagia, and diarrhea. In India, both Ayurveda and Unani medical systems use various parts of the plant for preventing uterine infections, as well as addressing issues like ulcers, bile problems, leprosy, fever, smallpox, jaundice, and piles.[13] For the treatment of kidney stones, several medicinal plants, including *Mimosa pudica*, offer potential benefits with fewer side effects. *Mimosa pudica leaf juice is commonly added to tea while the root powder is consumed before breakfast.*[14]

Zea Mays L. (corn silk) exhibits various pharmacological activities, including diuretic effects, hepatoprotective properties, anti-diabetic activities, antioxidant capacities, antibacterial effects, and antifatigue properties.[15] Traditional Chinese Medicine (TCM) refers to corn silk as "Yu Mi Xu," a substance rich in diuretic properties. TCM uses corn silk to treat urinary system ailments, including urinary tract infections and kidney problems.[16,17] Ayurveda traditionally uses corn silk for its diuretic and soothing properties, which are useful in promoting urinary tract health and addressing urinary tract problems such as bladder infections, kidney stones, and edema.[18,19]

The synergy among different herbs within a polyherbal formulation can theoretically produce better results than using each herb individually or even the sum of their individual effects. This phenomenon, known as synergism, plays a crucial role in ayurvedic formulations. Synergistic interactions between herbs are guided by their properties, and specific combinations are chosen to enhance their positive effects.[20]

In this research, calcium oxalate crystals were prepared to ensure precise control over their size, shape, and composition. This allows for the close mimicry of the physiological conditions under which kidney stones are formed and dissolved. *In vitro* aggregation and nucleation assays are crucial for assessing the ability of herbal formulation to prevent or reduce kidney stone formation. Nucleation assays determine how calcium oxalate crystals start to form, and aggregation assays determine how crystals tend to stick together. Both of these are important steps in the stone formation process. By analyzing the inhibitory effects of herbal tea on these stages, its potential efficacy in preventing and managing urolithiasis is determined. The primary objective of this research was to investigate the impact of novel herbal tea of a mixture of *Trigonella foenum-graecum*, *Mimosa pudica*, and *Zea mays* on kidney stone by assessing the quantity of calcium oxalate crystals obstructed in comparison to a control group in an *in vitro* experiment.

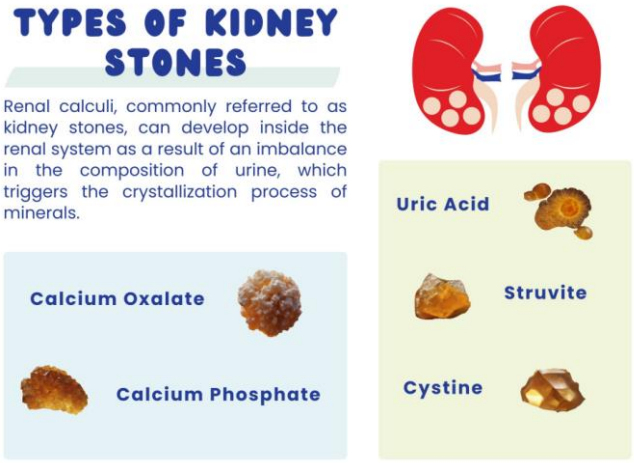

Figure 1: Types of kidney stones

Materials and Methods

Chemicals and Reagents

Calcium chloride dihydrate (SYSTERM ®, Malaysia), sodium chloride (SYSTERM ®, Malaysia), sodium oxalate (SYSTERM ®, Malaysia), Tris (Vivantis, Malaysia), 2N sulfuric acid (Sigma, Malaysia), dilute ammonia solution (R&M, Malaysia), distilled water, calcium chloride solutions, sodium oxalate solutions (Sigma, Malaysia).

Preparation of chemicals

A 2 N sulfuric acid solution was prepared by gently adding 5.439 mL of sulfuric acid to 25 mL of deionized water and the final volume was adjusted to 100 mL.

Preparation of Tea Bag

Fresh leaves of *Trigonella foenum-graecum* L, and *Mimosa pudica* L, were harvested in June 2023, while the stigma of *Zea mays* L. were harvested in August 2023, all from Cheras, Malaysia. The plant materials were dried, and thereafter pulverised using an electric blender. The powdered plant materials were weighed separately, and then mixed in the following ratios of *Trigonella foenum-graecum* Leaves:*Mimosa pudica* Leaves:*Zea mays* stigma; 50:25:25 for tea bag formulation 1, 25:50:25 for tea bag formulation 2, and 25:25:50 for tea bag formulation 3 (Table 1). The tea bags were then steeped in 100 mL of hot water.

Nucleation assay
Nucleation assay was performed using 0.6057 g of Tris and 0.8766 g of NaCl at pH 6.5. Briefly, to a 100 mL Tris buffer solution, 0.7351 g of $CaCl_2$ and 0.1005 g of $Na_2C_2O_4$ were dissolved. A sample solution containing $CaCl_2$ and tea extract (1 mL) were mixed at different concentrations ranging from 0.1 - 1.0 mg/mL [10 dilutions]). A standard solution of cystone (positive control) was also prepared in a similar manner as the test sample solution. The solutions were incubated at 37°C for 4 h. The absorbance of the incubated solutions was obtained using a UV spectrophotometer at a wavelength of 650 nm. All the experiments were done in triplicate and the percentage inhibition of calcium oxalate crystal formation was calculated using the formula:[21]

Percentage inhibition (%) = [(Abs of Control – Abs of Sample)/Abs of Control] x 100

Aggregation assay
Aggregation assay was performed using 0.6057 g of Tris and 0.8766 g of NaCl at pH 6.5. Briefly, 80 mg of calcium oxalate crystals was diluted in 100 mL of Tris buffer. Test sample solution of different concentrations (0.1 - 1.0 mg/mL) along with a standard drug cystone solution were prepared separately. The prepared solution was mixed with 3 mL of calcium oxalate solution in a test tube up to a total volume of 4 mL. The absorbance of the solution was measured using an UV spectrophotometer at 620 nm. The percentage inhibition of calcium oxalate formation was calculated using the formula below:[21]

Percentage inhibition % = [(Abs of Control – Abs of Sample)/Abs of Control] x 100

Microscopic analysis
After the nucleation and aggregation assays, all the samples were viewed under the light microscope (Motic, B1-253SP) to identify the shape, size and quantity of calcium oxalate present.

Statistical analysis
The results were expressed as mean ± SEM. Data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnette's multiple comparison test. A p-value of less than 0.05 was considered statistically significant.

Results and Discussion

Urolithiasis is a prevalent urological condition affecting the global population, with calcium oxalate and calcium phosphate being the most common types of stones. Diet, hydration, and hereditary factors can influence the combination of calcium and oxalate in the urine to form these stones. Urolithiasis can cause significant pain; therefore, understanding its prevalence and the types of stones commonly implicated is essential for prevention and treatment. Surgical and pharmaceutical interventions for urolithiasis possess disadvantages such as invasiveness, increased recurrence rates, adverse effects, financial strain, and pain during convalescence. Nonetheless, conventional medicine presents alternatives with potential advantages. Conventional diuretic and anti-inflammatory herbs promote renal health in a natural and holistic manner.[22] These treatments may also target underlying causes, reducing stone recurrence. The cultural acceptance, reduced side effects, and cost-effectiveness of traditional medicine render it a viable alternative for certain individuals. In the present study, an *in vitro* investigation using herbal tea combinations of *Trigonella foenum-graecum* leaves, *Mimosa pudica* leaves, and *Zea mays* hair was done to examine their pharmacological potential in preventing crystal development processes, including nucleation and aggregation.

Based on the nucleation assay, tea bags with higher concentrations showed higher inhibition calcium oxalate crystal formation compared to those with lower concentrations (Figure 2). All three tea bag formulations showed significant ($p < 0.05$) reduction in calcium oxalate crystal formation compared to the control group. Tea bag formulation 1 (50% *Trigonella foenum-graecum*, 25% *Mimosa pudica* , and 25% *Zea mays*) has a percentage inhibition of 94.96 ± 3.77%, tea bag formulation 2 (25% *Trigonella foenum- graecum*, 50% *Mimosa pudica* , and 25% *Zea mays*) has percentage inhibition of 91.34 ± 6.29%, formulation 3 (25% *Trigonella foenum-graecum*, 25% *Mimosa pudica* , and 50% *Zea mays*) has an inhibition rate of 93.24 ± 3.07%, while the positive control (cystone) has a percentage inhibition of 98.40 ± 1.65%. The Tea Bag formulation 1 demonstrated the highest suppression of calcium oxalate crystals formation at a concentration of 1.0 mg/mL.

The results obtained from the aggregation assay showed that all three tea bag formulations demonstrated a concentration-dependent increase in inhibitory effect on calcium oxalate crystal formation (Figure 3). At 1.0 mg/mL concentration, the inhibition of calcium oxalate crystals obtained for cystone, tea bag formulation 1, tea bag formulation 2, and tea bag formulation 3 are 81.71 ± 6.27%, 79.84 ± 1.66%, 75.74 ± 3.77%, and 73.27 ± 4.57%, respectively. All formulations significantly ($p < 0.05$) reduced calcium oxalate crystal formation compare to the control group. Among the three tea bag formulations, tea bag formulation 1 which consisted of 50% *Trigonella foenum-graecum*, 25% *Mimosa pudica*, and 25% *Zea mays* exhibited the highest inhibitory effect on calcium oxalate crystals aggregation.

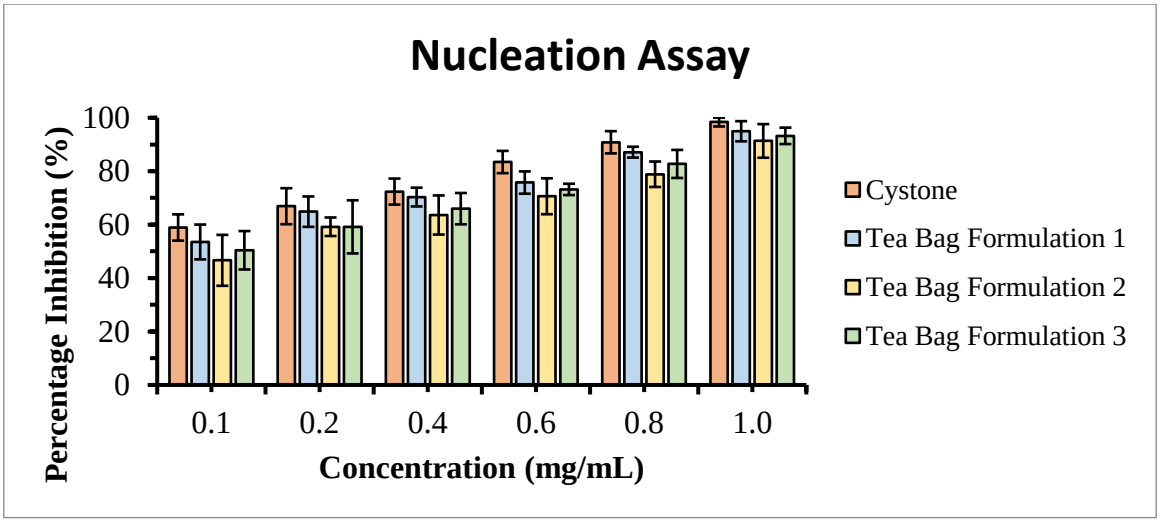

Figure 2: Percentage inhibition of calcium oxalate crystals after 4 hours incubation with herbal tea bag extract

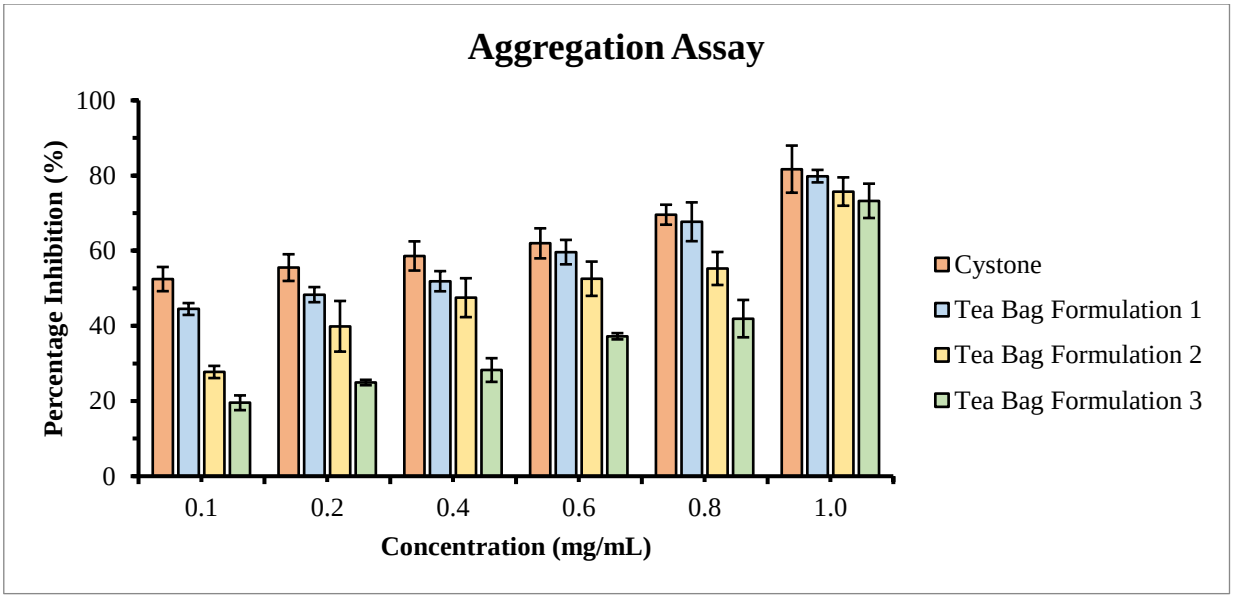

Figure 3: Percentage inhibition of calcium oxalate crystals aggregation

In the nucleation process, the solute particles present in the urine adjoin to form the initial nucleus of a crystal during crystallization.[23] Nucleation assay is performed in this study to evaluate the efficacy of the herbal tea formulations in inhibiting or promoting the nucleation process. Calcium chloride, and sodium oxalate were used to initiate the nucleation process. In this process, calcium ions are found to be much essential in building calcium oxalate crystals thus play a crucial role in the nucleation process of calcium oxalate crystals, while sodium oxalate serve as a source of oxalate ions to initiate nucleation. This interaction triggers the nucleation process, which results in the development of calcium oxalate crystals. The Tris buffer (pH 6.5) was used to maintain the physiological pH which is necessary for numerous biological systems, including the urinary tract where the production of kidney stones takes place. Biological systems typically exhibit an ideal pH range to ensure proper functioning. This pH value of 6.5 corresponds to the pH levels generally observed in specific body fluids, such as urine. This enhances compatibility with the biological samples used in the nucleation assay.[24] The turbidity of the tea extract in the calcium oxalate solution is then measured using a UV spectrophotometer.

Aggregation is the process where particles or substances combine to create larger clusters or aggregates. Therefore, it is an important aspect of kidney stone progression.[25] An aggregation assay can determine the rate of clustering in the crystal development process. Spectroscopic analysis at a wavelength of 620 nm can measure the degree of crystal aggregation. The absorbance at 620 nm may indicate the presence of a distinct absorption peak associated with calcium oxalate crystals. Different substances have the ability to absorb light at specific wavelengths, and calcium oxalate crystals has been shown to exhibit high absorbance at a wavelength of 620 nm. This wavelength was selected to minimize interference from other chemicals in the solution. This specific wavelength provides a precise signal directly associated with the absorption of calcium oxalate crystals. Therefore, spectroscopic analysis at this wavelength is used to assess the inhibitory potential of the herbal formulations against calcium oxalate crystals aggregation by comparing the absorbance value with that of the control. Light microscope (x10 objective) was used to observe the calcium oxalate crystals in each nucleation and aggregation assays for each concentration. Figure 4 shows that the tea extracts, when used in the nucleation and aggregation assays, effectively reduced the formation of calcium oxalate stones at 1.0 mg/mL compared to cystone, resulting in a decrease in both the crystal size and number of stones. It has been shown that some extracts could change calcium oxalate monohydrate crystals into calcium oxalate dihydrate crystals, which are less likely to stick to kidney epithelial cells. This suggest that such extracts might be beneficial in preventing kidney stones.[26] In both the nucleation and aggregation assays, tea bag formulation 1 exhibited the highest percentage calcium oxalate inhibition compared to the other tea bag formulations. This indicates its effectiveness in inhibiting calcium oxalate crystal production both in the nucleation and aggregation processes.

Table 1: Parts of herbal plants used

No.	Botanical name	Vernacular name	Family	Part used	Quantities
1.	*Trigonella foenum-graecum L.*	Fenugreek	Fabaceae	Leaves	100 g
2.	*Mimosa pudica L.*	Shameplant	Mimosaceae	Leaves	100 g
3.	*Zea mays L.*	Corn/Maize	Poaceae	Hair	100 g

The major herbal component in tea bag formulation 1 is *Trigonella foenum-graecum* (fenugreek) which constitute 50% of the total components. Fenugreek leaves contain significant phytochemicals, including saponins, flavonoids, tannins, alkaloids, fiber, amino acids,

steroids, mucilage, different vitamins, minerals, and phytosterols.[27] Saponins contained in fenugreek possess diuretic effects, leading to an increased urine output. The diuretic effect may reduce the concentration of minerals in the urine thereby lowering the probability of crystal formation.[28] Oxidative stress is a contributing factor that may lead to kidney stone formation. Polyphenols, encompassing flavonoids and tannins, provide antioxidant properties that mitigate oxidative stress and can dramatically reduce the formation of oxalate stones.[29] Additionally, fenugreek contains alkaloids that may have diuretic effect. This facilitates the excretion of surplus minerals via urine, hence reducing the chance of kidney stone formation.[30] The dietary fiber in fenugreek leaves may contribute to decreased mineral absorption in the digestive tract and regulate mineral concentration in urine.[31] Furthermore, diosgenin, a steroidal saponin present in fenugreek, exhibits anti-inflammatory properties that aid in alleviating the inflammation associated with urolithiasis.[32] The presence of mucilage, recognised for its gel-forming properties, may exert a calming effect on the urinary system, hence promoting overall kidney function.[33] Additionally, fenugreek contains phytosterols that may indirectly influence kidney function by modifying cholesterol metabolism.[34] Furthermore, the balanced composition of vitamins and minerals in fenugreek, including vitamin A, vitamin C, calcium, and potassium, enhances overall health and plays a vital role in the prevention of many types of stone formation.[35,36] Vitamin A is essential for maintaining healthy immune function, vitamin C has antioxidant properties that help combat oxidative stress, calcium is essential for maintaining appropriate calcium levels to potentially avert kidney stone formation, and potassium is essential for regulating fluid flow within the kidneys.[37,38]
The tea bag formulation 1 contains 25% *Mimosa pudica* leaves, which has been shown to contain phytochemicals such as flavonoids, saponins, tannins, alkaloids, flavonol, glycosides, mucilage, amino acids, terpenoids, and quinones.[39] Flavonoids and tannins help control inflammatory responses in the kidneys, and alkaloids and saponins possess diuretic properties, which helps the body get rid of extra minerals.[28,29]

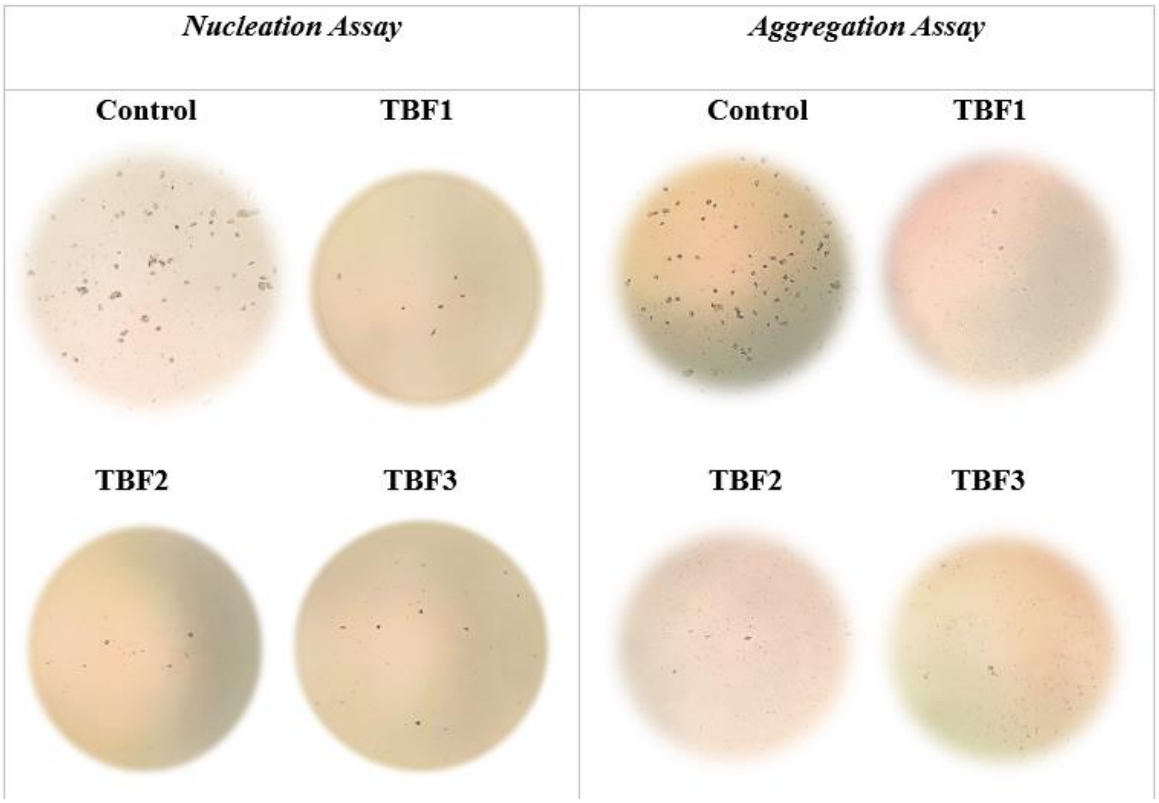

Figure 4: Micrographs of calcium oxalate crystals (magnification x10) of control, tea bag formulation 1 (TBF1), tea bag formulation 2 (TBF2) and tea bag formulation 3 (TBF3)

Flavonol glycosides are a distinct category of flavonoids recognised for their antioxidant effects. They assist in neutralizing free radicals within the body and mitigating oxidative stress, thus safeguarding kidney cells from damage induced by reactive oxygen species (ROS).[40] Phenolic compounds have the potential to mitigate kidney stone formation by directly addressing inflammation and oxidative stress, which are critical contributors to kidney stone development. Terpenoids in *Mimosa pudica* exhibit antioxidant properties. Terpenoids are abundant sources of anti-inflammatory compounds that mitigate kidney inflammation. Nephroprotective properties of terpenoids may arise from their powerful antioxidants and anti-inflammatory agents properties, which aid in preserving kidney health.[41] Quinones, existing in many forms, demonstrate multiple biological features. The distinct actions associated with kidney health may vary, although the many biological activities of quinones suggest a potential role in enhancing *Mimosa pudica*'s overall health benefits. Quinones may exhibit numerous advantageous characteristics, including antioxidant activities, anti-inflammatory actions, and various health-promoting attributes, contingent upon their precise classification.[42]
The husk of *Zea mays* L. (Poaceae) is utilized in Ibibio traditional medicine to cure numerous conditions, including diabetes mellitus, dyslipidaemia, and malaria.[18] Polysaccharides in maize silk may increase urine production, causing diuretic effects. Enhanced urine excretion may encourage the removal of surplus minerals from the body, potentially reducing the likelihood of crystal formation.[43] Flavonoids in maize silk have antioxidant effects, whereas terpenoids in maize silk possess anti-inflammatory properties similar to those in *Mimosa pudica* leaves. Corn silk contains vitamins and minerals that provide essential ingredients to enhance overall health. Optimal maintenance of metabolic processes, especially those related to renal function, requires adequate consumption of vitamins and minerals. Mucilage, a gel-forming substance, may offer a soothing impact on the

urinary system. This soothing effect can alleviate the discomfort associated with kidney stones.[18]

Researchers have investigated the combined efficacy of *Trigonella foenum-graecum* (fenugreek) leaves, *Mimosa pudica* (sensitive plant) leaves, and *Zea mays* hair (corn silk) in the treatment of urolithiasis through their individual bioactive constituents and their synergistic mechanisms of action. The combined diuretic properties of fenugreek, *Mimosa pudica*, and maize silk rid the body of excess minerals that could otherwise cause kidney stones. Flavonoids, tannins, and various other compounds found in the individual plant have antioxidant and anti-inflammatory effects, hence mitigating oxidative stress and inflammation associated with kidney stones. Research has shown that herbal medicines outperform conventional treatments, showing fewer side effects, cost-effective, and has no risk of recurrence. *In vitro* investigation of the impact of two plant species, *Mimosa pudica* and *Linum usitatissimum* on urolithiasis has demonstrated that the aqueous extracts of both plants have the ability to reduce the build-up of calcium oxalate in the kidneys, both when used alone and in combination with other plants. The combination of aqueous extracts of both plants demonstrated significant efficacy compared to the standard and control groups.[47] The present investigation also indicated that when used in conjunction with other medicinal plants, *Mimosa pudica* exert great inhibitory effect on calcium oxalate crystals, with above 70% inhibition. This suggests that mixtures of several herbs work better at breaking down calcium oxalate crystals.

The anti-urolithiatic activity of *Crataeva nurvala* bark, *Zea mays* (corn silk), the ayurvedic formulation Neeri tablet, as well as cystone (standard) was investigated *in vitro* using synthetic calcium oxalate crystals on a semi-permeable egg-shell membrane. The results indicated that the aqueous extract of corn silk exhibited a significant anti-urolithiatic effect by dissolving 60.42% of the calcium oxalate crystals. This efficacy was comparable to the ayurvedic formulation Neeri (59.52%) and the standard cystone (64.29%). The study concluded that corn silk aqueous extract demonstrated significant anti-urolithiatic activity.[45]

Previous studies have documented the anti-urolithiatic properties of numerous herbal plants. However, there is no documented research on the leaves of *Trigonella foenum-graecum*, the leaves of *Mimosa pudica*, or the hairs of *Zea mays*. Although, independent studies have documented the anti-urolithiatic action of each of these medicinal plants, there has not been much scientific research on the combined effect of these plant parts for the treatment of urolithiasis. Therefore, the present study shows that the new polyherbal mixture made of *Trigonella foenum-graecum* leaves, *Mimosa pudica* leaves, and *Zea mays* hair works synergistically against urinary tract stones by preventing the formation of calcium oxalate crystals. On the basis of these findings, further research, including clinical trials, is required to substantiate the potential advantages and determine the appropriate formulation and dosage for effectiveness. The shortcoming of the present study is the reliance on *in vitro* tests, which may limit direct clinical applicability. Therefore, there is the need for an *in vivo* study to provide a deeper understanding of the effects, and possibly the long-term adverse effects of the polyherbal formulation. Vast development in investigating the herbal plants as alternative therapeutic agents for different type of diseases including kidney stone [46]. Furthermore, an evaluation of the potential interactions with conventional urolithiasis therapies, and clinical trials are required for a comprehensive assessment of the efficacy of this herbal combination.

Conclusion

The findings from the present study have shown that the herbal tea blend of *Trigonella foenum-graecum* leaves, *Mimosa pudica* leaves, and the hairs of *Zea mays* may serve as a natural therapy for urolithiasis. This study demonstrated the effectiveness of the herbal tea blend in preventing calcium oxalate crystals formation or its aggregation in an *in vitro* model. Therefore, the herbal tea extracts provides a non-invasive solution to mitigate the pain and suffering associated with urolithiasis. Furthermore, the cost-effectiveness of the herbal tea provides additional advantage in alleviating the financial burden on urolithiasis patients, and the healthcare systems. However, further study is required to elucidate the mechanisms of action of the various components of the herbal tea, and to optimize the herbal formulation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Sultan S, Umer SA, Ahmed B. Urolithiasis. In: godbole P, Wilcox D, Koyle MA (Eds), Practical Pediatric Urology: An Evidence-Based Approach. Springer, 2023. 377–403 p.
2. Yousefichaijan P, Azami M, Ranjbaran M, Azami S, Rahmati S. Clinical Signs, Causes, and Risk Factors of Pediatric Kidney Stone Disease: A Hospital-Based Case-Control Study. Nephrourol Mon. 2017; 9(1):1-5.
3. Liu Y, Chen Y, Liao B, Luo D, Wang K, Li H, Zeng G. Epidemiology of urolithiasis in Asia. Asian J Urol. 2018; 5(4):205–214.
4. Perumal KR, Chua RHB, Teh GC, Lei CCM. Prevalence of urolithiasis in Sarawak and associated risk factors: An ultrasonagraphy-based cross-sectional study. BJUI Compass. 2023; 4(1):74–80.
5. Nouri AI and Hassali MA. Assessment of kidney stone disease prevalence in a teaching hospital. Afr J Urol. 2018; 24(3):180–185.
6. Vijaya T, Sathish Kumar M, Ramarao NV, Naredra Babu A, Ramarao N. Urolithiasis and Its Causes-Short Review. The J Phytopharmacol. 2013; 2(3):1–6.
7. Huang Y, Zhang YH, Chi ZP, Huang R, Huang H, Liu GY, Zhang Y, Yang H, Lin J, Yang T, Cao SZ. The Handling of Oxalate in the Body and the Origin of Oxalate in Calcium Oxalate Stones. Urol Int. 2020; 104(3–4):167–176.
8. Kasote DM, Jagtap SD, Thapa D, Khyade MS, Russell WR. Herbal remedies for urinary stones used in India and China: A review. J Ethnopharmacol. 2017; 203:55–68.
9. Kant R, Singh TG, Singh S. Mechanistic approach to herbal formulations used for urolithiasis treatment. Obes Med. 2020; 19:100266.
10. Ahmed S, Hasan MM, Mahmood ZA, Muhammad C, Hasan M, Mahmood A. Antiurolithiatic plants in different countries and cultures. J Pharmacogn Phytochem. 2016; 5(1):102–115.
11. Hinad I, S'hih Y, Elgui R, Mesfioui A, Elhessni A, Ouahidi ML. Acute and Subacute Toxicity Study of *Trigonella foenum-graecum.L* Seed Extract in Wistar Rat. Trop J Nat Prod Res. 2023; 7(10):4912-4915
12. Chatterjee S, Ghosh B, Chandra I. Fenugreek (*Trigonella foenum gracum* L.) and its necessity (A Review Paper Fenugreek (*Trigonella foenum-graecum* L.) and its necessity [Review Paper]. Fire J Eng Technol. 2015; 1(1):60-67.
13. Majeed I, Rizwan K, Ashar A, Rasheed T, Amarowicz R, Kausar H, Zia-Ul-Haq M, Marceanu LG. A Comprehensive Review of the Ethnotraditional Uses and Biological and Pharmacological Potential of the *Genus mimosa*. Int J Mol Sci. 2021; 22(14):7463.
14. Zahid IH, Bawazir AS, Naser R. Plant based native therapy for the treatment of Kidney stones in Aurangabad (M.S). J Pharmacogn Phytochem. 2013;1(6):189–193.

15. Tian S, Sun Y, Chen Z. Extraction of Flavonoids from Corn Silk and Biological Activities *In Vitro*. J Food Qual. 2021; 2021:1–9.
16. Revilla P, Alves ML, Andelković V, Balconi C, Dinis I, Mendes-Moreira P, Redaelli R, Ruiz de Galarreta JI, Vaz Patto MC, Žilić S, Malvar RA. Traditional Foods From Maize (*Zea mays* L.) in Europe. Front Nutr. 2021; 8:683399.
17. Wang H, Shi S, Wang S. Can highly cited herbs in ancient Traditional Chinese medicine formulas and modern publications predict therapeutic targets for diabetes mellitus? J Ethnopharmacol. 2018; 213:101–110.
18. Okokon JE, Nyong ME, Thomas PS, Daniel AO, Enin GN, Udobang JA. Antiobesity Activity of Extract, Fractions and Pure Compounds from Husk of *Zea mays*. Trop J Nat Prod Res. 2021; 5(10):1868–1875.
19. Middha SK, Usha T, Tripathi P, Marathe KY, Jain T, Bhatt B, Masurkar YP, Pande V. An *in vitro* studies on indigenous ayurvedic plants, having hypoglycemic activity. Asian Pac J Trop Dis. 2012; 2(SUPPL.1):S46–S49.
20. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. Pharmacogn Rev. 2014; 8(16):73.
21. Senthil Kumari C and Logeshwari B. *In silico* and *In vitro* Evaluation of Anti-Urolithiatic Activity of Ethanolic Extract of *Hybanthus enneaspermus* (Linn.) F. Muell. Int J Pharm Res Applic. 2021; 6(2):548-560.
22. Butterweck V and Khan SR. Herbal medicines in the management of urolithiasis: alternative or complementary? Planta Med. 2009; 75(10):1095–1103.
23. Vekilov PG. Nucleation. Cryst Growth Des. 2010; 10(12):5007–5019.
24. Della Vecchia NF, Luchini A, Napolitano A, D'Errico G, Vitiello G, Szekely N, d'Ischia M, Paduano L. Tris Buffer Modulates Polydopamine Growth, Aggregation, and Paramagnetic Properties. Langmuir. 2014; 30(32):9811–9818.
25. Alelign T and Petros B. Kidney Stone Disease: An Update on Current Concepts. Adv Urol. 2018; 2018:1–12.
26. Hewagama SP and Hewawasam RP. Antiurolithiatic Potential of Three Sri Lankan Medicinal Plants by the Inhibition of Nucleation, Growth, and Aggregation of Calcium Oxalate Crystals *In Vitro*. The Sci World J. 2022; 2022:1–13.
27. Singh N, Yadav SS, Kumar S, Narashiman B. Ethnopharmacological, phytochemical and clinical studies on Fenugreek (*Trigonella foenum-graecum* L.). Food Biosci. 2022; 46:101546.
28. Ma ZN, Li YZ, Li W, Yan XT, Yang G, Zhang J, Zhao LC, Yang LM. Nephroprotective Effects of Saponins from Leaves of *Panax quinquefolius* against Cisplatin-Induced Acute Kidney Injury. Int J Mol Sci. 2017; 18(7):1407.
29. Ahmed S, Hasan MM, Khan H, Mahmood ZA, Patel S. The mechanistic insight of polyphenols in calcium oxalate urolithiasis mitigation. Biomedicine & Pharmacotherapy. 2018; 106:1292–1299.
30. Rashid S, Sameti M, Alqarni MH, Abdel Bar FM. *In vivo* investigation of the inhibitory effect of Peganum harmala L. and its major alkaloids on ethylene glycol-induced urolithiasis in rats. J Ethnopharmacol. 2023; 300:115752.
31. Sorensen MD, Hsi RS, Chi T, Shara N, Wactawski-Wende J, Kahn AJ, Wang H, Hou L, Stoller ML, Women's Health Initiative Writing Group. Dietary Intake of Fiber, Fruit and Vegetables Decreases the Risk of Incident Kidney Stones in Women: A Women's Health Initiative Report. J Urol. 2014;192(6):1694–1699.
32. Nunes CDR, Barreto Arantes M, Menezes de Faria Pereira S, Leandro da Cruz L, de Souza Passos M, Pereira de Moraes L, Vieira IJC, Barros de Oliveira D. Plants as Sources of Anti-Inflammatory Agents. Molecules. 2020; 25(16):3726.
33. Golkar P, Allafchian A, Afshar B. *Alyssum lepidium* mucilage as a new source for electrospinning: production and physicochemical characterisation. IET Nanobiotechnol. 2018; 12(3):259–263.
34. Patil AG, Prakash JK, More SS, Chandramohan V, Zameer F. Exploring Banana phytosterols (Beta-sitosterol) on tight junction protein (claudin) as anti-urolithiasis contributor in Drosophila: A phyto-lithomic approach. Inform Med Unlocked. 2022; 29:100905.
35. Ferraro PM, Mandel EI, Curhan GC, Gambaro G, Taylor EN. Dietary Protein and Potassium, Diet–Dependent Net Acid Load, and Risk of Incident Kidney Stones. Clin J Am Soc Nephrol. 2016; 11(10):1834–1844.
36. Han H, Segal AM, Seifter JL, Dwyer JT. Nutritional Management of Kidney Stones (Nephrolithiasis). Clin Nutr Res. 2015; 4(3):137.
37. Bargagli M, Ferraro PM, Vittori M, Lombardi G, Gambaro G, Somani B. Calcium and Vitamin D Supplementation and Their Association with Kidney Stone Disease: A Narrative Review. Nutrients. 2021; 13(12):4363.
38. Ferraro PM, Curhan GC, Gambaro G, Taylor EN. Total, Dietary, and Supplemental Vitamin C Intake and Risk of Incident Kidney Stones. Am J Kidney Dis. 2016; 67(3):400–407.
39. Lakshmibai R. Preliminary Phytochemical Analysis and Antioxidant Activities of Prosopis Juliflora and Mimosa Pudica Leaves. Int J Sci Eng Technol Res. 2015; 4(30):5766–5770.
40. Zeng X, Xi Y, Jiang W. Protective roles of flavonoids and flavonoid-rich plant extracts against urolithiasis: A review. Crit Rev Food Sci Nutr. 2019; 59(13):2125–2135.
41. Al-Mamoori F and Aburjai T. Perspective Chapter: Medicinal Plants for the Treatment of Nephrolithiasis. In: Nephrolithiasis - From Bench to Bedside. IntechOpen; 2023. Available from: http://dx.doi.org/10.5772/intechopen.108495
42. Madeo J, Zubair A, Marianne F. A review on the role of quinones in renal disorders. Springerplus. 2013; 2(1):139.
43. Zou GJ, Huang WB, Sun XY, Tang GH, Ouyang JM. Carboxymethylation of Corn Silk Polysaccharide and Its Inhibition on Adhesion of Nanocalcium Oxalate Crystals to Damaged Renal Epithelial Cells. ACS Biomater Sci Eng. 2021; 7(7):3409–3422.
44. Naga Kishore R. Investigation of Anti-Urolithiatic Activity of *Linum usitatissimum* and *Mimosa pudica* in Experimentally Induced Urolithiasis in Animal Models. Kishore World J Pharm Res. 2018; 7(1):1086-1092.
45. Bhandari R, Panta A, Panth P, Aryal D, Gautam U, Joshi DR, Kumar R, Rokaya TB, Pandey J, Aryal P. *In-vitro* Comparison of Anti-urolithiatic Effect of *Crataeva nurvala*, *Zea mays* (Corn Silk) and Ayurvedic Formulation Neeri Tablet. Int J Pharm Sci Rev Res. 2023; 66(1):26–30.
46. Khoo LW, Audrey Kow S, Lee MT, Tan CP, Shaari K, Tham CL, Abas F. A Comprehensive Review on Phytochemistry and Pharmacological Activities of *Clinacanthus nutans* (Burm.f.) Lindau. Evid Based Complement Alternat Med. 2018;2018:9276260.