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In Vitro Evaluation Of Urolithiasis Potential For A Combination Therapy Of Aerva Lanata And Citrus Medica

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ABSTRACT

The present study was to evaluate the in vitro antiurolithiatic potential of aqueous extract of Aerva lanata and aqueous extract of Citrus medica. The objective was to assess their individual and combined efficacy in dissolving calcium oxalate crystals, which are the primary cause of the kidney stones. Artificial kidney stone crystals were prepared by reacting calcium chloride with sodium oxalate. Aerva lanata powder and Citrus medica powder were extracted and prepared in different concentrations. These were tested separately and in combination with calcium oxalate crystals, and their dissolution capacity was observed under a microscope over specified incubation periods. The highest concentration of aqueous extract of Aerva lanata (7 mg/ml) dissolved 90% of the crystals, and aqueous extract of Citrus medica (300 mg/kg) dissolved 80%. The combination of both extracts showed the most significant effect, dissolving 99% of the crystals in vitro. These results indicate a strong synergistic action between the two herbal components in reducing calcium oxalate crystals. The study concludes that the combined herbal formulation of aqueous extract of Aerva lanata and aqueous extract of Citrus medica effectively reduces calcium oxalate crystals, indicating potential for kidney stone treatment. Further clinical trials are needed to confirm its therapeutic efficacy and safety in humans.

INTRODUCTION

History and Definition of Kidney Stone

Kidney stone disease, or nephrolithiasis, has been recognized for centuries, with archaeological evidence showing that humans have long suffered from kidney and bladder stones [1]. Historically, bladder stones were more common, particularly in earlier centuries, but kidney stones have become more prevalent over the past 100 years, especially in developed countries [2]. In ancient times, stone disease often had severe outcomes due to the lack of effective treatment, frequently resulting in death [3]. Early treatment methods such as bladder lithotomy and transurethral removal were painful and associated with high risk [4]. The incidence of urolithiasis has always been influenced by geographic, racial, socio-economic, and dietary factors [5]. In recent decades, changes in lifestyle and nutrition have significantly impacted the prevalence, composition, and location of kidney stones [6]. Calcium oxalate stones are now the most common type, often forming on calcium phosphate deposits known as Randall's plaques on the renal papillae [7]. Differences in dietary habits and infection rates explain the varying occurrence of uric acid and struvite stones across populations [8]. Kidney stones form when urine becomes supersaturated with certain minerals, leading to the development of mineral deposits in the renal calyces and pelvis [9]. These stones consist of both crystalline and organic materials [10]. The condition is highly prevalent, affecting up to 14.8% of the population, with recurrence rates as high as 50% within five years [11]. Risk factors include obesity, diabetes, hypertension, and metabolic syndrome, all of which are also linked to chronic kidney disease and even end-stage renal disease [12]. Management has evolved significantly, shifting from open surgery to minimally invasive endourological techniques, resulting in lower patient morbidity and better outcomes [13]. Preventing recurrence remains a major challenge and involves a combination of behavioral changes, dietary adjustments, and pharmacological interventions tailored to the stone type [14]. Nutritional factors play a critical role in both the development and prevention of kidney stones [15]. A better understanding of the underlying mechanisms is essential for developing more effective preventive therapies and treatments, highlighting the ongoing need for research in this field [16].

Kidney Stone Pathogenesis

The formation of hard, mineralized deposits in the renal tubules, known as nephrolithiasis or kidney stone disease, is a complex process [17]. No single theory can fully explain the pathogenesis of kidney stones due to their varied nature [18]. Based on human biopsies, imaging, and physiological data from ten stone-forming groups, three major pathways of stone formation have been identified [19]. The first involves overgrowth on interstitial apatite plaques, seen in idiopathic calcium oxalate stone formers and those with conditions like primary hyperparathyroidism or ileostomy [20]. The second pathway involves crystal deposits within renal tubules, observed in all groups except idiopathic calcium oxalate formers [21]. The third is free solution crystallization, evident in patients with cystinuria or

hyperoxaluria, often linked to obesity-related bypass surgery [22]. Although final stone composition may appear similar, their mechanisms of formation differ greatly, making it essential to accurately reflect human

conditions when developing experimental or animal models of nephrolithiasis [23].

Figure No. 1 Etiology of Kidney Stone [24]

Precautions

Aerva lanata:

- Pregnant and nursing women should avoid it as its safety has not been proven.
- Before using, speak with your doctor, particularly if you are taking diuretics or other medications, or if you already have liver or kidney issues.
- Use in moderation: Because of its diuretic effect, high dosages can cause excessive urination or electrolyte imbalance.
- Check for allergies: Some people may have allergic reactions; if you have itchy or rashes, stop using the product [25].

Citrus medica:

- Because of its high citrus content, high dosages may irritate or acidify the stomach.
- Steer clear of direct sunlight after using this product because it contains compounds like Bergapten that can make skin more sensitive to sunlight.
- To avoid allergic reactions, people who are allergic to citrus fruits should not use this product.
- See a doctor if you are taking medication. Certain medications, particularly those that are metabolized by the liver (such as statins or antihypertensives), may interact with citrus medica [25].

Current Treatments on The Nephrolithiasis

New Drug therapy

By addressing underlying metabolic abnormalities, recent developments in urolithiasis medication therapy aim to prevent stone formation and recurrence. For calcium stones, new treatments include Potassium Citrate and Thiazide diuretics; for uric acid stones, they include Allopurinol or Febuxostat. New treatments that target oxalate metabolism include RNA-based medications like Nedosiran, which lowers hepatic oxalate production, and probiotics that break down oxalate. Crystal growth inhibitors and citrate analogues are also being studied. In order to improve outcomes and lower recurrence in patients with recurrent kidney stones, these innovative therapies seek to customize treatment based on individual risk factors and stone composition [18].

Surgical Treatment

Urolithiasis can now be treated surgically with much more effective and less invasive options. Common procedures include ureteroscopy, which uses a thin scope to locate and remove or laser- fragment stones, and extracorporeal shock wave lithotripsy (ESWL), which uses sound waves to break stones into passable fragments. With percutaneous nephrolithotomy (PCNL), a tiny incision is made to remove the stone directly from larger or more complicated stones. When compared to open surgery, these techniques shorten recovery times and minimise complications. In order to achieve complete stone removal and symptom relief, the procedure chosen will depend on the size, composition, and location of the stone [26].

Herbal Remedies Treatment

For centuries, urolithiasis has been treated and prevented with herbal remedies. Plants with diuretic, anti-inflammatory, and stone-dissolving qualities include Boerhaavia diffusa, Tribulus terrestris, and Phyllanthus niruri, also referred to as "stone breaker." By changing the composition of the urine and decreasing crystal aggregation, these herbs may help reduce the size of stones, facilitate passage, and stop them from coming back. In addition to traditional treatments, herbal teas and extracts are frequently used as supportive therapies [27].

Targeted Microtubules

By interfering with the cellular mechanisms involved in crystal growth and retention within the kidneys, targeting microtubules in urolithiasis seeks to prevent the formation of stones. Through cellular-level intervention, microtubule-interfering agents can decrease tubular damage and crystal-cell adhesion, providing a potential therapeutic approach to reduce the development and recurrence of stones [28].

Table No. 1 List of Herbs Adopted from Ayurveda Pharmacopoeia of India Part I–VI, 2004–2009

Sr. No.	Botanical name	Family	Sanskrit name	Part/ used plant	Dose/ Mode
1	*Aerva lanata* (L.) Juss.	Amaranthaceae	Gorakshaganga, Astmabayda, Bhadra, Pashanabheda, Pattura	Whole plant	50-100 ml, Decoction

2	*Anisomeles malabarica* (L.) R. Br. ex Sims	Lamiaceae	Sprkka	Whole plant	3-5 g, Powder
3	*Apium graveolens* L.	Apiaceae	Karaphsa	Root	5-7 g, Powder
4	*Aerva lanata* L.	Amaranthaceae	Bhadra	Leaves	2-4 g, Powder
5	*Citrus medica* L.	Rutaceae	Matulunga	Juice	2-4 ml, Juice

LITERATURE SURVEY

Alsmadi J.K. et al., 2024 described that the field of urology has significantly evolved from traditional open stone removal surgeries to minimally invasive techniques such as mini- percutaneous nephrolithotomy (mini-PCNL) and retrograde intrarenal surgery (RIRS). In their comparative study, minimally invasive methods demonstrated shorter operative times, reduced hospital stays, fewer complications, and improved postoperative recovery compared to traditional approaches. The success rate of minimally invasive techniques reached 92%, outperforming the 85% observed in open procedures. Additionally, patients undergoing these procedures experienced an average hospital stay of 2.5 days, notably shorter than the 4.2 days associated with open surgeries. Despite their benefits, these modern techniques present challenges such as higher costs and the need for specialized training. However, Alsmadi J.K. et al. 2024 emphasized that the long-term patient-centered outcomes justify these hurdles. Their findings support a paradigm shift towards minimally invasive methods in stone management, offering superior clinical efficiency and aligning with the principles of modern, patient-focused care [13].

Abufaraj M. et al., 2021 described that the prevalence of kidney stone disease in the USA has shown a significant gender-specific trend over the last decade. Analyzing data from the National Health and Nutrition Examination Survey (NHANES) from 2007 to 2018, they found that while the prevalence among men remained relatively stable, it increased notably in women, particularly those under 60 years of age. The study highlighted those factors such as obesity, metabolic syndrome, gout, and female reproductive characteristics like multiple pregnancies, menopause, and hormone therapy were significantly associated with a higher prevalence of kidney stones. Additionally, non-hispanic white individuals showed a consistently higher prevalence compared to other ethnicities. These findings suggest shifting patterns in kidney stone epidemiology, with lifestyle, hormonal, and metabolic factors contributing to the gender gap closure. The authors emphasized the need for gender-specific preventive strategies to address the rising burden of nephrolithiasis, especially among women in the United States [6].

Cornu J.N. et al., 2016 described that endourological procedures, widely adopted for conditions such as benign prostatic obstruction (BPO), bladder tumors, and urolithiasis, are considered minimally invasive but still carry a notable risk of complications. These include bleeding, infection, ureteral injury, and urinary retention. The incidence and type of complications vary depending on the procedure, patient condition, and surgeon experience. Monopolar transurethral resection of the prostate (TURP) has been associated with higher bleeding and transurethral resection syndrome (TUR) rates, while laser-based techniques like holmium laser enucleation of the prostate (HoLEP) show reduced complications. Cornu J.N. et al. 2016 emphasized that complication reporting is inconsistent due to lack of standardized classification systems. They highlighted the importance of surgical expertise, proper equipment selection, and adherence to clinical guidelines in reducing risks. Additionally, management strategies like antibiotic prophylaxis, irrigation control, and morcellation technique optimization were noted as essential for complication prevention. The authors called for improved data reporting and procedural standardization to enhance patient safety [4].

Tefekli and Cezayirli , 2013 described that the history of urinary stones parallels human civilization, with early records from ancient Egypt and Mesopotamia. They noted that Hippocrates recognized bladder stones and warned against surgical removal due to its high risk. Sushruta in ancient India pioneered surgical removal through perineal lithotomy. Albucasis (930–1013 AD) improved surgical techniques by inventing new instruments and emphasizing lateral incisions to minimize damage. During the Renaissance, innovations like suprapubic lithotomy and sound-guided incisions emerged. By the 19th century, developments in lithotripsy began with Civiale's instrument to crush stones and Bigelow's "Litholopaxy" technique. The 20th century saw the rise of minimally invasive approaches such as ureteroscopy and percutaneous nephrolithotomy. The introduction of extracorporeal shock wave lithotripsy (ESWL) in 1980 revolutionized treatment. The evolution of these methods significantly reduced morbidity and surgical intervention, marking a shift toward non-invasive management of urolithiasis [1].

Eccles S.A. et al., 2013 described that despite significant advances in breast cancer research, several critical gaps hinder its effective prevention and treatment. In a comprehensive gap analysis, the authors highlighted the need for better understanding of genetic and epigenetic influences, improved risk prediction models, and more effective chemo preventive and lifestyle strategies. They emphasized that although genome-wide association studies (GWAS) have uncovered numerous genetic risk factors, nearly 50% of heritability remains unexplained. Epigenetic markers are emerging as valuable tools in risk assessment and early detection but require further validation. The authors also pointed out the lack of standardized methods for stratified screening and the necessity for translational research to convert discoveries into clinical applications. Lifestyle interventions, such as diet, weight control, and exercise, though promising, lack robust implementation frameworks. The study urged a multidisciplinary approach combining molecular biology, bioinformatics, and clinical trials to close these research gaps and enhance personalized breast cancer care [16].

Popova et al., 2025 described the innovative GeoBioMed approach, which conceptualizes kidney stones as biogenic minerals and applies geological methods to study their structure and formation. The authors emphasized the high recurrence rate of kidney stones and the need for a deeper understanding of their mineralogical and mechanical properties. Techniques like micro- CT, scanning electron microscopy, and polarized light microscopy were highlighted for their ability to analyze porosity, crystalline architecture, and fracture patterns. Thin-section petrographic analysis was proposed as a diagnostic tool, capable of revealing the chronological sequence of crystal growth and metabolic changes. The study also introduced AI and radiomics integration for automated stone classification and predictive modeling. Ultimately, the paper advocated for personalized treatment strategies and the development of clinical decision support systems based on stone morphology and patient-specific factors [23].

PLANT PROFILE

Aerva lanata:

Taxonomy:

Kingdom: Plantae (Plants)

Sub-kingdom: Tracheobionta (Vascular plants) Division: Magnoliophyta (Angiospermes, flowering plants)

Class: Magnoliopsida (Dicotylédones)

Subclass: Caryophyllidae Order: Caryophyllales Genus: Aerva

Species: Aerva lanata (L.) A. L. Juss. ex Schultes

Synonym: Chaya (Hindi), Gorakh Ganga (Hindi), Bili suli gida (Kannada), Koolappoo (Tamil), Polaippoo (Tamil), and Pashanabedha (Sanskrit)

Common Name: Paashaanabheda

Biological Source: Aerva lanata also known as Gorakha Ganga or Mountain Knotgrass, is a perennial shrub belonging to the family Amaranthaceae. It is native to tropical regions of Africa, Asia, and parts of the Indian Ocean area.

Family: Amaranthaceae

Geographical Sources: Sri Lanka, Tropical Africa, Australia, India (Assam, Karnataka, Maharashtra, Odisha, Rajasthan, Tamil Nadu, And Uttar Pradesh).

Morphology

An erect or prostrate herbaceous plant, Aerva lanata has many hairy, striated branches and a long taproot. Cottony hairs cover the undersides of alternating, elliptic to round leaves. The tiny, greenish-white, sessile, frequently bisexual flowers are grouped in dense spikes or axillary heads.

Phytochemical Constituents

The medicinal plant Aerva lanata is well-known for its diverse range of phytochemicals. Its antioxidant and anti-inflammatory qualities are attributed to a number of flavonoids, such as kaempferol, quercetin, isorhamnetin, and apigenin. Alkaloids with medicinal properties, including alkaloid glycosides, are also found in the plant. Shade-dried plant material contains saponins and phenolic compounds that can be extracted and have antimicrobial and diuretic properties. Its antiurolithiatic action is further supported by sterols such as ß-sitosteryl palmitate, α-amyrin, and ß-sitosterol. In addition, the plant has oil, proteins, carbohydrates, and tannins, all of which enhance its overall therapeutic potential.

Phytochemistry

Alkaloids: Aerva lanata contains biological active canthin-6-one alkaloids such as 10- methoxy- canthin-6-one, 10-hydroxy-canthin-6-one, 10-O-ß-D-glucopyranosyloxycanthin-6- one, 10-hydroxycanthin-6-one (ervine), 10-methoxycanthine-6-one (methylervine), 10-ß-D- glucopyranosyloxycanthin-6-one (ervoside), aervine (10-hydroxycanthin 6-one), methylaervine (10-methoxycanthin-6-one) and aervoside (10-ß-D-glucopyranosyloxycanthin- 6-one). Plant also contains alkaloids like ß-carboline-1 -propionic acid, 6-methoxy-ß carboline- 1-propionic acid, 6-methoxy-ß-carbolin-l-ylpropionic acid (ervolanine), and aervolanine (3-(6- methyoxy-ß- carbolin-1-yl) propionic acid).

Flavanoids: Aerva lanata is a rich source of flavonoids such as kaempferol, quercetin, isorhamnetin, isorhamnetin 3-O-ß-[4-p-coumaroyl-α-rhamnosyl(1→6) galactoside and flavanone glucoside persinol, persinosides A and B, 5, 4'-hydroxy-3, 6, 7-trimethoxyflavone, 5- hydroxy-3, 6, 7, 4-tetramethoxyflavone, 5-hydroxy-2,3,5,6,7-pentamethoxyl flavone, 3,3',5,7- trihydroxy-4'-methoxyflavone, apigenin 7-O-ß-D- glucoside and 7-O-ß-D-glucopyranoside.

Miscellaneous Phytoconstituents:

Aerva lanata also contains methyl grevillate, lupeol, lupeol acetate benzoic acid, ß-sitosteryl acetate and tannic acid.

Nutritive Content:

Leaves of Aerva lanata is highly rich in carbohydrate (26.6 g/100 g), crude protein (22.6 g/100g) and ash (31.2g /100g). The leaves of Aerva lanata is high in mineral composition (mg/100g) such as PO4 (187), and moderately high in other minerals such as Potassium (39.4), Calcium (51.7), Magnesium (41.5), Zinc (44.7), Ferrous (11.0) and low in Manganese (1.04).

Uses

There are many traditional uses for the valuable medicinal herb Aerva lanata. Because of its anti- inflammatory qualities, it works well to treat a variety of inflammatory conditions and reduce swelling. It supports urinary tract health and increases urine flow as a diuretic, particularly in the treatment and prevention of kidney stones. Because of its calming properties, it is also used to treat skin conditions like burns, rashes, eczema, and wounds. In terms of digestive health, it eases indigestion and constipation. Because of its expectorant qualities, the plant may help with respiratory conditions like cough and asthma. Aerva lanata contains antioxidant compounds that help fight oxidative stress and antimicrobial activity that may help fight infections. It is also utilised in conventional methods to treat diabetes, though further validation from science is required. All things considered, Aerva lanata supports many facets of health in traditional medicine [29].

Structure

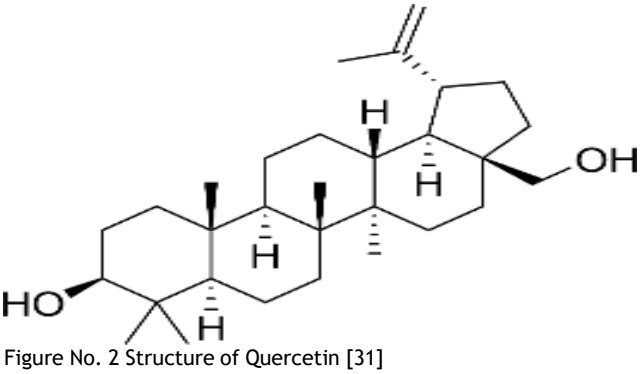


Figure No. 2 Structure of Quercetin [31]

Citrus medica:

Taxonomy/Biological Classification: Kingdom: Plantae (Plants)

Sub-kingdom: Tracheobionta (phylum plants)

Division: Angiospermes

Class: Magnoliopsida , Magnoliatae

Subclass: Rosidae Order: Sapindales Genus: Citrus

Species: Citrus medica L.

Synonym: Citrus alata (Tanaka) , Citrus bicolor (Poit. and Turpin) , Citrus cedar (Link), Citrus fragrans (Salisb) , Citrus limonimedica (Lush.), Citrus odorata (Wester) Tanaka, Citron, Wild Lemon, Bijapura, Limbu, Nimbu.

Common Name: Mahalungi

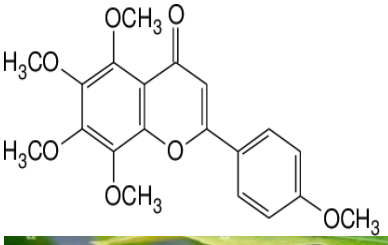


Figure No. 3 Citrus medica Fruit [33

Biological Source: A big, fragrant citrus fruit with a thick skin of Citrus medica belonging to the family Rutaceae and is believed to have come from Southeast Asia, especially the eastern parts of Malaysia, China, and India.

Family: Rutaceae

Geographical Source:

Nepal And Bhutan, Indian (Tamil Nadu, Maharashtra, Uttar Pradesh, Himachal Pradesh, Bihar, Sikkim, Meghalaya, Tripura, Arunachal Pradesh).

Morphology

An evergreen shrub or small tree reaching 4 meters tall, with purplish, smooth young branches and straight axillary spines up to 4 cm long. Leaves are simple, alternate, spirally arranged with margined petioles (0.5–1 cm). Blades are oblanceolate or obovate (6–18 × 3–9 cm), gland- dotted, with a rounded base, acute apex, and crenate-serrate edges. Secondary veins occur in 8– 12 pairs. Axillary racemes bear few white or pinkish flowers (1.5 cm), which are bisexual or staminate, with a 4–5 lobed urceolate calyx, 5 petals, and 30–40 polyadelphous stamens. The ovary has 12–14 locules, a 15 mm purplish style, and a sticky pink stigma. Fruits are yellow (10– 20 × 6–14 cm), aromatic, with a hard whitish mesocarp and 10–12 pale green, acidic or sweet segments. Seeds are smooth and white internally.

Phytochemicals

Citrus medica contains flavonoids like apigenin, hesperetin, hesperidin, naringin, naringenin, rutin, quercetin, and diosmin , terpenes like limonene, γ-terpinene, limonin, and nomilin , phenolic acids like p-coumaric acid, trans-ferulic acid, and chlorogenic acid , coumarins like citropten, scoparone, and bergapten and other compounds like citral, linalool, decanal, nonanal, pectin, and heteropolysachharides.

Uses

a. Aids digestion, has anti-inflammatory, antioxidant, and antibacterial properties, and supports respiratory health.

b. Used for flavouring, making marmalade, or candied peels.

c. Essential oil used in perfumes, aromatherapy, and as an insect repellent.

d. Natural tonic for acne and skin tightening.

Structure:

Figure No. 4 Structure of Tangeritin [34]

MATERIALS AND METHODS

Material

a. Aerva Latana plant and Citrus medica fruit were collected from Sonai village, Tal. Newasa, dist. Ahilyanagar (MH). Calcium chloride, sodium oxalate, methyl paraben, propyl paraben, distilled water and hydrochloric acid, orange essence were procured from the department of pharmacognosy at KBHSS Trust's Institute of Pharmacy, Malegaon, dist. Nashik (MH). The access of using hot air oven and stability testing machine was taken from the department of Pharmaceutics at KBHSS Trust's Institute of Pharmacy, Malegaon, dist. Nashik (MH).

Method:

Preparation of Artificial Kidney Stone Crystals

1.47 g of calcium chloride was dissolved in 100 ml of distilled water, while 1.34 g of sodium oxalate was dissolved in 100 ml of 1 M H_2SO_4. The sodium oxalate solution was then added slowly to the calcium chloride solution with continuous stirring for 10–15 minutes. The resulting mixture was left undisturbed for 2–3 days to allow crystallization to occur. After this period, crystals were formed and collected. Microscopic observation revealed that the crystals were rod-shaped in structure.

Preparation of Aqueous Extract of Aerva lanata

Fresh Aerva lanata leaves were collected, dried, powdered using a mortar and pestle, and passed through sieve no. 60. The powder was mixed with water, boiled, concentrated, and the residue was collected and dried to obtain a semisolid extract. This extract was redissolved in water, stirred to form a homogeneous solution, and filtered. Three concentrations of aqueous extract (3 mg/ml, 5 mg/ml, and 7 mg/ml) were prepared and each was treated with 2 mg/ml of calcium oxalate crystals. The mixtures were incubated for 24 hours to evaluate their activity. It was observed that the 7 mg/ml concentration showed the highest efficacy, dissolving about 90% of calcium oxalate crystals.

Aqueous Extract of Citrus medica

Citrus fruits were washed, and the peel (flavedo) along with the inner white layer (albedo) was removed. The fruit was sliced, seeds were removed, and the juice was extracted, filtered, and dried to obtain a powder, which was further subjected to long-term drying to yield a semi-solid residue. This extract was used to prepare three concentrations of aqueous extract of Citrus medica [A (200 mg/kg), B (250 mg/kg), and C (300 mg/kg)]. Each sample was mixed with 2 mg/ml of calcium oxalate crystals and incubated at 37°C for 24 hours. The results showed that sample C (300 mg/kg) exhibited the highest activity, dissolving approximately 80% of calcium oxalate crystals.

For Blank Sample:

Calcium oxalate (CaC_2O_4) crystals were added to purified water to prepare a test sample, which was labeled as sample A. This sample served as a control (blank) to observe any natural changes in the absence of treatment. The mixture was kept under similar conditions as other test samples. Upon observation, no visible change or dissolution of calcium oxalate crystals was noted. This confirmed that purified water alone had no effect on the crystals.

Mixture: Aqueous Extract of Aerva lanata (AEAL) and Aqueous

Extract of Citrus medica (AECM) Preparation

The aqueous extracts of Aerva lanata and Citrus medica were mixed together, filtered, and a homogeneous mixture was obtained. Calcium oxalate (CaC_2O_4) crystals (2 mg/ml) were then added to this mixture at different concentrations in three test tubes: A (AEAL 3 mg/ml + AECM 200 mg/kg), B (AEAL 5 mg/ml + AECM 250 mg/kg), and C (AEAL 7 mg/ml + AECM 300 mg/kg). The

samples were incubated for 12 hours under controlled conditions. After incubation, significant dissolution of calcium oxalate crystals was observed in all samples. The highest activity was seen in test tube C, where approximately 99% of the crystals were dissolved.

Preparation of Syrup IP For 100 ml:

The simple syrup base was prepared according to the Indian Pharmacopoeia guidelines. A solution containing 30 mg of aqueous extract of Aerva lanata and 10 ml of aqueous extract of Citrus medica was prepared, mixed, and filtered to obtain a clear filtrate. This filtrate was then incorporated into the syrup base with continuous stirring. Preservatives, namely methyl paraben and propyl paraben, were added to enhance stability. The mixture was stirred for about 10 minutes to achieve a homogeneous formulation. Finally, the prepared syrup was filled into an airtight container for storage.

Formulation:

Table No. 2 Formulation Table of Herbal Syrup IP (Renalix Syrup)

Sr. No.	Ingredients	Quantity (100 ml)	Category
1	Aqueous Extract of *Aerva lanata*	30 mg	Antiurolithatic
2	Aqueous Extract of *Citrus medica*	10 ml	Antiurolithatic
3	Methyl Paraben	0.15 g	Preservative
4	Propyl Paraben	0.05 g	Preservative
5	Syrup Base	q.s	Simple Syrup
6	Orange Essence	1-2 drop	Flavouring Agent

RESULT AND DISCUSSION

Prepared Artificial Kidney Stone Crystals

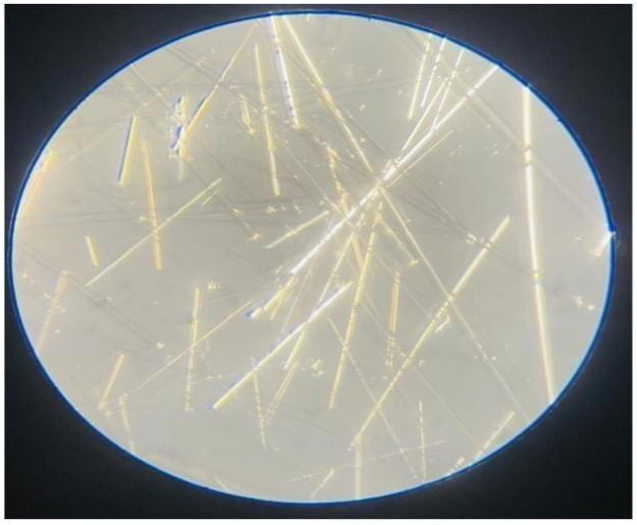


Figure No. 5 Prepared Calcium Oxalate Crystals

The cylindrical road shape long crystals were observed under the simple microscope with the help of 10x lens. In the human body, it can form kidney stones, a condition known as urolithiasis. Most artificial kidney stones are made up of calcium oxalate.

Prepared Aerva lanata Powder

Figure No. 6 Aerva lanata Powder

The dried powder of Aerva lanata (L.) was extracted from whole shrub. The collected powder was fine, clean, greenish in colour and also uniform in nature. It is traditionally used in ayurvedic medicine, its dried leaves and stems are ground into a powder which are used to treat urolithiasis (kidney stone).

Prepared Citrus medica Juice:

Figure No. 7 Citrus medica Juice

The aqueous extract of Citrus medica was obtained after cleaning, grinding and then finely crushed the citrus fruit.

Observation of Blank Solution:

Figure No. 8 Observation of Blank Solution Sample

Figure No. 9 Observation of Blank under microscope

Calcium oxalate crystals were artificially prepared in the laboratory and dissolved in purified water at a concentration of 2 mg/mL in varying volumes in three different test tubes (A, B, C). After the in-vitro study of kidney stones, it was observed under a simple microscope that the sample in test tube C contained crystalline, transparent, rod-shaped crystals. Therefore, this sample concentration was selected for further analysis.

Table No. 3 Observation Table of Blank Sample

Concentration	A	B	C
Observation	No change	No change	No change

Observation of Aerva lanata Sample:

Figure No. 10 Observation of *Aerva lanata* sample

Figure No.11 Observation of Sample C *Aerva lanata* Sample under microscope

Three Aerva lanata solutions were prepared by dissolving the powder in water, stirring, and filtering to obtain a concentrated extract for in vitro testing. Three different concentrations [A (3 mg/ml), B (5 mg/ml, C (7 mg/ml)] of the extract solution were taken in three separate test tubes. After in vitro study, sample C was observed under the microscope, where 2 to 3 crystals were seen as shown in the figure no.14.

Table No. 4 Observation Table of Aerva lanata Sample

Concentration	A (3 mg/ml)	B (5 mg/ml)	C (7 mg/ml)
Observation	Poorly dissolve	Slightly dissolve	Completely dissolve

Observation Of Citrus medica Sample:

Figure No. 12 Observation of *Citrus medica* Sample

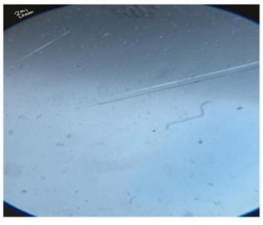


Figure No. 13 Observation of Sample C *Citrus medica* Sample Under Microscope

Citrus medica juice was filtered and used to prepare three test samples [A (200 mg/kg), B (250 mg/kg), C (300 mg/kg)], each mixed with 2 mg/ml of calcium oxalate crystals. Upon microscopic examination, sample C showed the presence of two crystals. This sample was then selected for further microscopic observation to evaluate its crystal dissolution potential.

Table No. 5 Observation Table of Citrus medica Sample

Concentration	A (200 mg/kg)	B (250 mg/kg)	C (300 mg/kg)
Observation	Poorly dissolve	Slightly dissolve	Completely dissolve

For Mixture:

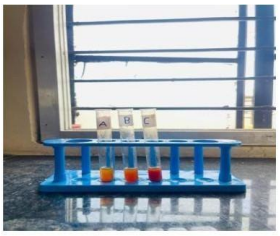


Figure No. 14 Observation of Mixture

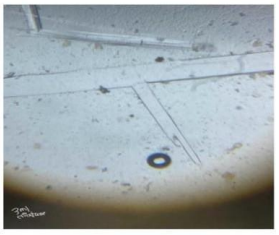


Figure No. 15 Observation of Sample C Mixture under microscope

Aerva lanata and Citrus medica are the main ingredients, which were mixed in a 1:1 ratio to prepare the final formulation. This mixture was used to create three test samples A,B,C with concentrations of

a. Test tube A : AEAL 3 mg/ml and AECM 200 mg/kg
b. Test tube B : AEAL 5 mg/ml and AECM 250 mg/kg
c. Test tube C : AEAL 7 mg/ml and AECM 300 mg/kg

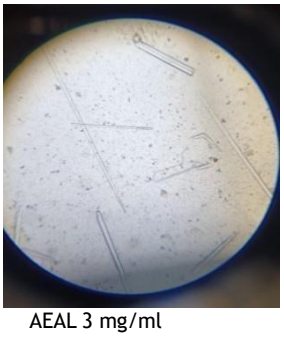
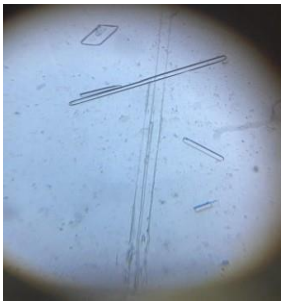
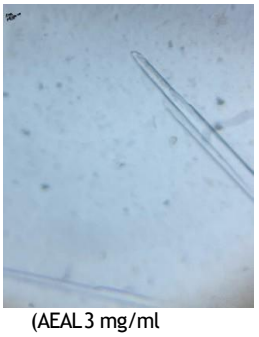
Each sample was treated with quantity 2 mg/ml of calcium oxalate crystals. Upon microscopic observation, the C (AEAL 7 mg/ml and AECM 300 mg/kg) sample showed the highest crystal dissolution.

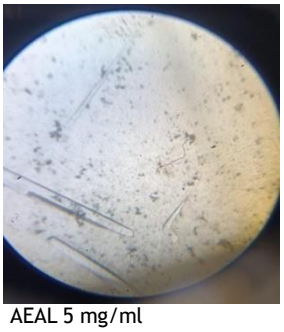
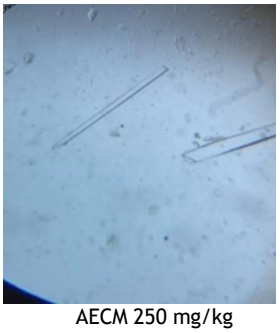
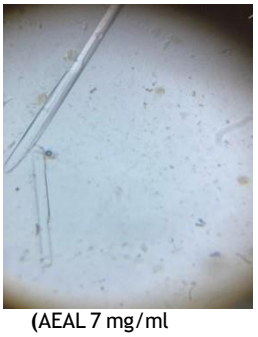
Figure No. 16 Herbal Syrup IP (Renalix Syrup)

Table No. 6 Observation Table of Mixture

Test Tube No.	A	B	C
Concentration	(AEAL 3 mg/ml and AECM 200 mg/kg)	(AEAL 5 mg/ml and AECM 250 mg/kg)	(AEAL 7 mg/ml and AECM 300 mg/kg)
Observation	Poorly dissolve	Slightly dissolve	Completely dissolve

Table No.7 Observation Table of Samples Observed Under the Microscope

Test	Blank	*Aerva lanata*	*Citrus medica*	Mixture(*Aerva lanata* + *Citrus medica*)
A	 Calcium oxalate Crystals 2mg/ml	 AEAL 3 mg/ml	 AECM 200 mg/kg	 (AEAL 3 mg/ml and AECM 200 mg/kg)

B	----------	 AEAL 5 mg/ml	 AECM 250 mg/kg	 (AEAL 5 mg/ml and AECM 250 mg/kg)
C	----------	 AEAL 7 mg/ml	 AECM 300 mg/kg	 (AEAL 7 mg/ml and AECM 300 mg/kg)

Based on the above observations, it was concluded that the sample C was consistently more effective in all cases. Therefore, Aerva lanata, Citrus medica, and their mixture at this concentration were selected for the final formulation, in comparison to the A and B concentrations.

Table No. 8 Evaluation Parameters

Sr.no.	Test	Observation	Inference
A. Physical Test			
a.	**Colour**	Dark brown	Compiles
b.	**Odour**	Citrusy	Compiles
c.	**Taste**	Sweet citrusy	Compiles
B. Evaluation Test			
1.	**pH**	5.0 (4.0 - 5.5 Optimum range)	pH was found in optimum range
2.	**Viscosity**	100 – 300 cP	Viscosity was found in optimum range

3.	**Specific Gravity**	1.25	Evaluated
4.	**Clarity**	Slightly turbid	Evaluated
5.	**Stability**	No change	Formulation was stable

CONCLUSION

The formulated herbal syrup IP (Renalix Syrup), combining aqueous extract of Aerva lanata and aqueous extract of Citrus medica, offers a promising natural remedy for the management of urolithiasis. The diuretic, anti-inflammatory, and antioxidant properties of Aerva lanata, along with the citrate-rich, crystallization-inhibiting effects of Citrus medica, work synergistically to reduce kidney stone formation, promote dissolution, and enhance urinary flow. Preliminary in vitro findings support its effectiveness in reducing stone size and improving renal function. herbal syrup IP (Renalix Syrup) represents an innovative integration of traditional herbal knowledge with modern pharmaceutical formulation, providing a safe and effective alternative for kidney stone treatment. Further research, including comprehensive clinical trials, is necessary to validate its therapeutic potential, ensure safety, and standardize dosage for human use.

FUTURE PERSPECTIVE

The promising in vitro results of the combination therapy using Aerva lanata and Citrus medica suggest its potential as a viable alternative or adjunct to conventional urolithiasis treatments. Future studies should focus on in vivo evaluations to validate efficacy, pharmacokinetics, and safety. Clinical trials and mechanistic studies are essential to determine optimal dosage and understand the molecular pathways involved. Enhancing formulation stability and bioavailability will aid in commercial viability. This herbal combination could offer a cost-effective, accessible treatment for kidney stones.

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