International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 8, 2025
https://www.theaspd.com/ijes.php

Stoneease: A Synergistic Herbal Syrup With Citrus Medica Linn., Rubia Cordifolia, And Bryophyllum Pinnatum For Natural Kidney Stone Relief

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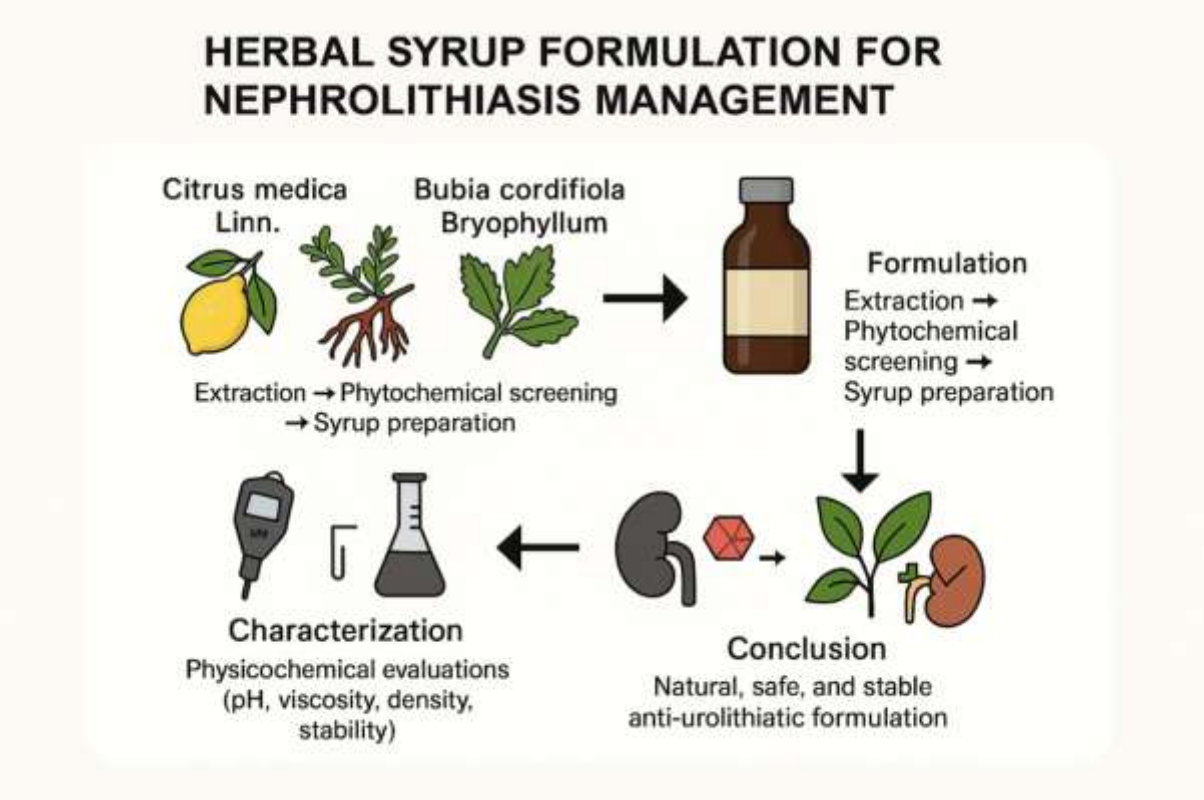
GRAPHICAL ABSTRACT:



ABSTRACT:

Nephrolithiasis, commonly known as kidney stones, is a prevalent urinary tract disorder affecting nearly 12% of the global population, with over 600,000 new cases reported annually in India. These stones are typically composed of calcium oxalate and calcium phosphate, forming when urinary mineral concentration exceeds solubility limits. Conventional treatment approaches aim to adjust urinary composition through dietary or pharmacological modifications. The present study focuses on the development and assessment of a novel polyherbal syrup formulated with Citrus medica Linn., Rubia cordifolia, and Bryophyllum pinnatum, all recognized for their anti-urolithiatic, antioxidant, and anti-inflammatory effects. In particular, Citrus medica contains high levels of citric acid, which may help prevent crystal formation by binding calcium and increasing urinary pH. The syrup was formulated as a sugar-based aqueous oral preparation, ensuring patient compliance and enhanced palatability. Key formulation parameters such as pH, viscosity, specific gravity, and density were systematically evaluated. The anti-urolithiatic potential was investigated through in vitro dissolution studies mimicking kidney stone breakdown. Furthermore, the stability of the herbal formulation was analyzed following ICH guidelines to ensure its shelf life and efficacy. This study highlights the therapeutic promise of combining traditional herbal knowledge with contemporary formulation science in addressing nephrolithiasis. The results indicate that this herbal syrup could serve as a viable, natural alternative to conventional treatments, contributing to improved renal health and patient well-being.

Keywords: *Kidney stone, Medicinal plant, Nephrolithiasis, Bijora fruit, Herbs*

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1. INTRODUCTION:

Nephrolithiasis

Nephrolithiasis, commonly referred to as kidney stones, stands as the most prevalent disorder affecting the urinary system, impacting approximately 12% of the global population and afflicting 600,000 individuals in India annually. This condition manifests as the formation of crystal or crystalline concretions within the genitourinary system subsequent to their passage from the kidneys[1]. Nephrolithiasis, a multifactorial urological ailment characterized by kidney crystal concretion, poses an increased risk of end-stage renal failure, affecting 12% of the world's population[2]. These mineral deposits, situated in the renal calyces and pelvis, either free or adherent to the renal papillae, are commonly termed kidney stones. Their genesis occurs when urine becomes supersaturated with respect to a particular mineral, encompassing both organic and crystalline components. Predominantly composed of calcium oxalate, many stones develop on the surface of renal papillaries, where calcium phosphate deposits, known as Randall's plaques, are prevalent[3]. These hardened deposits of minerals and salts, forming within the kidneys, are commonly known as kidney stones or nephrolithiasis, increasingly prevalent and indicative of various underlying conditions, including endocrinopathies, metabolic syndrome, and genetic disorders[4].

Epidemiology

Approximately 1 in 11 Americans experience the impact of kidney stones, serving not only as a manifestation of systemic illness but also as a predictor of metabolic and cardiovascular complications[4].

Pathophysiology

The precipitation and crystallization of urinary solutes within the urinary space lead to the formation of kidney stones. Stone formation can arise from genetic predispositions, metabolic abnormalities, or environmental factors.

Management

The management of kidney stones involves identifying urine chemistries predisposing individuals to stone formation and subsequently modifying these risk factors through dietary adjustments or pharmaceutical interventions.

Types of kidney stones

Deviations in the composition of urine's various substances dictate the chemical makeup of kidney stones, resulting in variations in their size, shape, and mineral composition (mineralogy)[5]. Kidney stones are commonly classified into five categories, each distinguished by differences in their mineral composition and pathophysiology[6]. Figure 1 illustrates the types of nephrolithiasis.

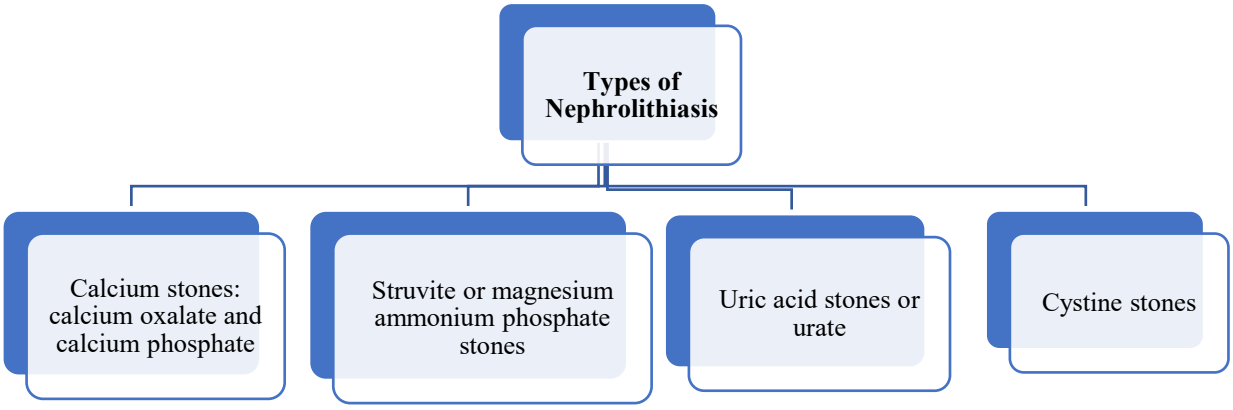

Fig. 1 Types of Kidney Stones

Calcium stones: Calcium oxalate & Calcium phosphate

Calcium oxalate and calcium phosphate are the predominant constituents of urinary calculi, collectively constituting approximately 80% of all kidney stones. Among these, calcium phosphate (CaP), also known as apatite, accounts for 5% of calcium stones, while pure calcium oxalate (CaOx) comprises 50%, with a combination of both types making up the remaining 45%. Brushite, alternatively referred to as hydroxyapatite or calcium hydrogen phosphate, serves as the primary component of calcium stones. Kidney stones commonly contain calcium oxalate in the form of CaOx monohydrate (COM, also known as weddellite, $CaC_2O_4 \cdot H_2O$) or CaOx dihydrate (COD, also known as whewellite, $CaC_2O_4 \cdot 2H_2O$), or often a mixture of the two. Among these, COM represents

the most thermodynamically stable form, prevalent in clinical stones more frequently than COD. The formation of CaOx stones is multifactorial, influenced by various conditions including hyperuricosuria, hyperoxaluria, hypocitraturia, hypomagnesuria, hypercystinuria, and hypercalciuria, which encompasses resorptive, renal leak, absorptive, and metabolic disorders. Conversely, calcium phosphate stones tend to develop in environments where the urine pH exceeds 7.5, while CaOx stones are more likely to form within a pH range of 5.0 to 6.5. It is noteworthy that calcium stones exhibit a higher recurrence rate compared to other types of kidney stones[7].

Struvite or magnesium/ ammonium phosphate stones

Struvite stones, composed of magnesium ammonium phosphate, represent a significant subset of kidney stones. These stones often form in response to urinary tract infections caused by urease-producing bacteria, such as Proteus mirabilis, which hydrolyze urea to ammonia, increasing the urine pH and promoting stone formation. Struvite stones can grow rapidly and become quite large, potentially filling the renal pelvis and calyces, a condition known as staghorn calculi. These stones typically form in alkaline urine (pH > 7.0) and are more common in women due to the higher incidence of urinary tract infections in this population. Management of struvite stones often involves treating the underlying infection with appropriate antibiotics, as well as surgical removal of the stones. Preventative measures include maintaining good urinary hygiene and, in recurrent cases, the use of urease inhibitors[7].

Uric acid stones or urates

Uric acid stones account for approximately 3–10% of all kidney stones. These stones are primarily associated with high purine diets, which are rich in animal proteins like meat and fish, leading to hyperuricosuria (excessive uric acid in the urine), low urine volume, and a persistently low urine pH (pH < 5.5). These conditions facilitate the formation of uric acid crystals and subsequent stone formation. Uric acid stones are particularly common in individuals with gouty arthritis and those with metabolic syndrome. Men are more prone to developing uric acid stones than women, and idiopathic nephrolithiasis is often the most common cause. Management strategies for uric acid stones include dietary modifications to reduce purine intake, increasing fluid intake to enhance urine volume, and alkalinizing the urine with medications like potassium citrate to dissolve the stones and prevent recurrence.

Cystine stones

Cystine stones are rare, constituting less than 2% of all kidney stones. These stones result from a hereditary disorder known as cystinuria, which affects the renal tubular reabsorption of cystine and other dibasic amino acids. Due to a mutation in the rBAT gene on chromosome 2, affected individuals excrete excessive amounts of cystine in the urine. Cystine is poorly soluble in urine, leading to the formation of cystine stones. Individuals with cystinuria excrete over 600 millimoles of insoluble cystine daily, significantly increasing the risk of stone formation. The primary clinical manifestation of cystinuria is the recurrent formation of cystine stones, which can occur as early as childhood. Management includes increasing fluid intake to dilute the urine, dietary restrictions to reduce cystine excretion, and medications like tiopronin or D-penicillamine, which bind to cystine and increase its solubility. Urine alkalinization with potassium citrate is also beneficial in preventing stone formation

Herbal Syrup

Fig. 2 Herbal Syrup

Aqueous formulations that are concentrated and can be medicated or non-medicated are called syrups. Their main ingredient is sugar or sugar substitutes mixed in water, sometimes together with other flavorings and medications. Syrups' high sugar content gives them sweetness and viscosity, which can help mask the flavor of any added medication. Pharmaceutical companies frequently employ syrups to make their products more palatable, especially for young patients[8,9].

There is different type of syrups, including: -

Simple syrup: Contains only sugar and purified water.

Medicated syrup: Contains therapeutic agents for treatment purposes.
Flavoured syrup: Contains flavouring agents but no medicinal substances.
Traditionally, citron, or *Citrus medica Linn.*, has been employed in a variety of medical formulations. Due to its bioactive components, it has particularly fascinating potential as a medicated Active Pharmaceutical Ingredient (API) in syrups for kidney stone therapy. These components include phenolic acids, terpenes, coumarins, and flavonoids, which have demonstrated beneficial characteristics such as anti-inflammatory and antioxidant properties[10].
Citrus medica Linn. may be beneficial for kidney stones because of its citric acid content, which can raise urine citrate levels and potentially aid in stone prevention. Citric acid is known to bind to calcium in the urine, reducing the likelihood of calcium stone formation. Furthermore, citric acid's alkalinizing action can help raise the pH of urine, which may also prevent certain types of kidney stones.[11].
It's crucial to remember that although citrus juices, such as those from oranges and grapefruits, have been linked in some studies to a protective effect against the development of kidney stones, they have also been associated with higher levels of oxalate in the urine, which may increase the risk of stone formation.
Consequently, it is important to carefully evaluate and design the usage of Citrus medica Linn. in kidney stone syrups to optimize its therapeutic benefits and minimize any potential risks[12]. Research suggests that Citrus medica Linn. has anti-bacterial and anti-struvite crystal properties, indicating that it might be developed as a potential therapy for kidney stones, especially those caused by specific bacteria like Proteus mirabilis. As a result, it has potential for use as a medicinal active ingredient in syrups intended to treat or prevent kidney stones[13].

2. MATERIALS AND METHODS

Citrus medica linn. **(Bijora fruit):**
The bijora fruit from Rutaceae family has citral as main chemical constituent[14]. It is mainly used for inhibition the spontaneous nucleation and agglomeration of calcium oxalate crystals i.e. Anti-naphrolithiasis activity[15].

Boerhaavia diffusa **(Punarnava):**
Punarnava is from Nyctaginaceae family has Liriodendrin as main chemical constituent[16]. It helps to **pass the stone through urine by increasing urine flow. This is due to its diuretic and anti-inflammatory** property[17].

Bryophyllum pinnatum **(Akkapan):**
Akkapan is from Crassulaceae family has Astragalin & 2-nonenal as main chemical constituent[18]. Several uses of it are **diuresis, dissolving kidney stones, Anti-inflammatory & Analgesic activity**[19].

Rubia cordifolia **(Manjistha):**
Manjistha is from Rubiacea family has Daucosterol & Beta-sitosterol as main chemical constituent[14]. Main use of it is to **reduce growth of urinary crystals & diuretics**[20].

Honey:
Honey is used from ancient time. Dextrose is main chemical constituent of it. It is used as **Antioxidant & Sweetening agent.** It can also use as **Vehicle** in syrup preparation[21].

Acacia catechu **(Cutch):**
Catechu is from Leguminosae family has Catechin as main chemical constituent. It has **Preservative activity**[21,22].

Method of Preparation
The preparation process begins with the extraction of the drug powders using the decoction method. First, 31.25 grams each of *Citrus medica Linn., Boerhavia diffusa, and Bryophyllum pinnatum* drug powders are separately dissolved in 500 ml of distilled water. These mixtures are heated until only one-fourth of the original volume remains, after which they are filtered to complete the extraction process, making the extracts ready for use. Next, the juices are prepared. For *Citrus medica Linn.* (bijora fruits), the fruits are cut into pieces, placed in a mixer to make juice, and then filtered using Whatman filter paper. Similarly, for *Bryophyllum pinnatum* (Akkapan juice), the leaves are cut into pieces, processed in a mixer to produce juice, and filtered using Whatman filter paper.
Finally, for the preparation of the medicated syrup, the decoctions and juices are combined according to the specified ratios in the formulation table. This mixture is heated until a homogeneous mixture

is achieved. After cooling, preservatives are added as outlined in formulation table no. 1 and 2. This method ensures that the active ingredients are effectively extracted and integrated into the syrup, preserving the therapeutic properties of the herbal components[23].

Fig. 2 Bijora Fruit Juice

Fig. 3 Decoction Process

Fig. 4 Filter Process of Decoction

Table No. 1: Formulation Batches (F1-F7)

Ingredients	F1	F2	F3	F4	F5	F6	F7
Citrus medica linn. (Bijora fruit)	20%	20%	20%	20%	20%	20%	20%

Rubia cordifolia (Manjishtha)	20%	20%	20%	20%	20%	20%	20%
Boerhavia diffusa (Punarnava)	20%	10%	20%	20%	18%	17%	15%
Bryophyllum pinnatus (Akkapana)	20%	10%	20%	20%	20%	20%	20%
***Accasia catechu* (Catechu)**	-	-	-	-	-	-	-
Honey	-	20%	-	20%	20%	20%	20%
Simple syurp	20%	20%	20%	-	-	-	-
Sodium salt of methyl paraben	-	-	-	-	2 %	3 %	5 %
Orange peel powder	-	-	-	-	-	-	-
Inference	Smelling issue and Bacterial infection was observed	Bacterial infection was observed	Particle separation issues & fungal infection was observed	2 layers were separated.	Fungal infection was observed	Fungal infection is still not resolved	Bacterial infection and smell issue remain stable

Table No.2: Formulation batches (F8-F14)

Ingredients	F8	F9	F10	F11	F12	F13	F14
Citrus medica linn. (Bijora fruit)	20 %	20 %	25 %	20 %	25 %	26.6 %	26.6 %
Rubia cordifolia (Manjishtha)	20 %	20 %	20 %	20 %	17 %	20 %	20 %
Boerhavia diffusa (Punarnava)	13 %	-	-	-	-	-	-
Bryophyllum pinnatus (Akkapana)	20 %	20 %	20 %	20 %	17 %	23.4 %	20%
***Accasia catechu* (Catechu)**	-	-	-	-	-	3.3 %	6.7%
Honey	20 %	20 %	25 %	17 %	-	23.4 %	20 %
Simple syrup	-	-	-	-	-	-	-
Orange peel powder	7 %	-	-	6 %	20.5 %	-	-
Sodium salt of methyl paraben	-	20 %	10 %	17 %	20.5 %	3.3 %	6.7%
Inference	Fungal infection and smell issue was observed	Smelly issue was resolved	smelly issue was resolved but stability issue was observed	Sodium salty MP. preserve syrup	Formulation was Stable.	Herbal syrup is prepared	Herbal syrup is prepared.

Evaluation parameters

The characterization of herbal syrup includes Organoleptic characteristics, Viscosity, Density, and Specific Gravity, following standard guidelines given in the Indian Pharmacopoeia (IP).

Viscosity: To determine the viscosity, thoroughly clean the Ostwald viscometer with warm chromic acid, and if necessary, use an organic solvent such as acetone. Mount the viscometer in a vertical position on a suitable stand. Fill the viscometer with water up to mark G and count the time required for water to flow from mark A to mark B, repeating this step at least three times to obtain an accurate reading. Rinse the viscometer with the test liquid, fill it up to mark A, and record the time required for the liquid to flow to mark B. Additionally, determine the densities of the liquid as mentioned in the density determination experiment[24].

Viscosity is calculated using the formula:

$$\textbf{Viscosity} = \frac{\text{Density of test liquid} \times \text{Time required to flow test liquid} \times \text{Viscosity of water}}{\text{Density of water} \times \text{Time required to flow water}} \quad ...(1)$$

Density: Clean the specific gravity bottle thoroughly with chromic acid or nitric acid, rinse it at least two to three times with distilled water, and if required, rinse it with an organic solvent like acetone and dry it. Take the weight of the empty dry bottle with the capillary tube stopper (w_1). Fill the bottle with the unknown liquid, place the stopper, and wipe out excess liquid from the outside of the tube using tissue paper. Weigh the bottle with the unknown liquid on an analytical balance (w_2) and calculate the weight in grams of the unknown liquid (w_3).

The weight of the liquid under test (w_3) is calculated as:

Weight of liquid under test (w_3) = Weight of bottle with liquid under test (w_2) – Weight of empty dry bottle (w_1).

Considering the 25 ml volume of liquid due to the fixed volume of the bottle, the density of the liquid syrup under test is calculated as[24].

$$\textbf{Density of liquid syrup under test (syrup)} = \frac{\text{Weight of liquid under test } (w_3)}{\text{Volume of liquid under test}} \quad ...(2)$$

Specific Gravity: Clean the specific gravity bottle thoroughly with chromic or nitric acid, rinse it at least two to three times with purified water, and if required, rinse it with an organic solvent like acetone and dry it. Take the weight of the empty dry bottle with the capillary tube stopper (w1). Fill the bottle with distilled water, place the stopper, wipe out excess liquid from the side tube using tissue paper, and weigh the bottle with stopper and water on an analytical balance (w_2). Repeat the procedure for the liquid under test by replacing the water after emptying and drying as mentioned in the previous steps. Weigh the bottle with the stopper and the liquid under test on an analytical balance (w_3).

The weight of the liquid under test is calculated as

Weight of liquid under test=Weight of liquid under test with bottle (w_3)—Empty dry bottle (w_1)[24]

The specific gravity is then calculated as

$$\textbf{Specific gravity} = \frac{\text{Weight of liquid under}}{\text{Weight of water}} \quad ...(3)$$

***In-vitro* Study:** The prepared syrup is used as an experimental medium to evaluate variations in the weight of renal calculi. The procedure to perform the in-vitro study is as follows: After the preparation of the syrup, it is transferred to a glass Erlenmeyer flask. Calculi are placed in a porous bag and submerged in the syrup at ambient temperature. For each experiment, the weight loss of the renal calculi is evaluated by weighing the calculus after drying it in an oven at 40°C for 18 hours, 24 hours, and 48 hours. The activity of the syrup is assessed by calculating the rate of calculi dissolution over time, comparing their residual weight to their initial weight before the study. The percentage of dissolution is calculated using the following formula, with results shown in Figure and Table 3:

$$\textbf{a}\% = \frac{(W_{initial} - W_{final})}{W_{initial}} \times 100 \quad ...(4)$$

a% = is the rate of calculi dissolution; W initial and W final are the weights of the calculus before and after the incubation with the plant-based syrup[25].

Stability

Tastings:

The primary objective of short-term stability studies is to evaluate the drug product under accelerated conditions, typically at a temperature of 40°C ± 2°C and a relative humidity (RH) of 75% ± 5%. These conditions are designed to stress the product and help identify potential degradation pathways and rates.

The standard duration for these studies is six months, during which samples are evaluated at multiple time points, commonly at 0 (initial), 1, 2, 3, and 6 months.
Several critical parameters are assessed during the stability study. These include physical characteristics (such as appearance, color, and odor), pH (especially for liquid formulations), microbial limits (for non-sterile products), and water content (as syrup may lose water and you will observe recrystallization). Thorough documentation of all observations and results is essential, covering methodology, test results at each time point, and any deviations from standard procedures[26].

Microbial Limit Test

Membrane filtration technique was performed to quantify microbial contamination of the collected syrup samples. This involved passing the samples through filter nitrocellulose membrane disks with a pore size of 0.45 µm under biological safety cabinet in order to collect any potential contaminants (bacteria or fungi) on the filter membrane disk. The following steps were performed according to the ***European pharmacopoeia 2010***: after preparing the media (TSA and SDA), two dilutions (1/10 and 1/100) were made of each syrup (using physiological serum as the diluent) in order to reach a dish with a density of countable colonies. After filtering the samples, the membrane filter disks were washed three times with hundred milliliters of sterile physiological serum. Each membrane was then placed on the surface of a dish of culture media so that the germ carrying face is up while avoiding air reservation between the membrane and the middle surface. When the dishes were incubated, the germs pulled nutrients through the membrane and formed colonies. Three replicate dishes were cultured of each dilution along with the original sample concentration. TSA dishes were incubated at 35 °C for 2 days in the bacterial incubator for the detection of bacterial growth and SDA dishes were incubated in the fungal incubator at 25 °C for 7 days for the detection of fungal growth. Colonies were counted and the number of viable cells in the original sample and in each dilution was expressed as colony forming units per milliliter (CFU/mL). Colony forming units per milliliter were calculated using the following equation. CFU/mL = CFU/plate × dilution factor.

Detection of *E. Coli*

Syrups were analyzed according to the ***European pharmacopoeia 2010*** with additional steps for the presence and absence of *E. coli*. 10 ml of each syrup was passed through a filter membrane which was then washed three times with sterile physiological serum to eliminate the efficacy of the preservative and any excipients that might have an antimicrobial effect. Each membrane was then cultured in a tube containing sterile MacConkey broth as an enrichment medium. The tubes were then incubated for 48 h at 35 °C. Subsequently, by the culture loop after inflaming it, an extract was taken from each tube of a particular syrup and spread on three EMB agar plates and three MacConkey agar plates. Following a 48-hour incubation at 35 °C, the plates were visually examined to confirm each syrup was free from *E. coli* through the shape, specifications and color of the colonies after incubation. If green metallic shine colonies appeared on the surface of EMB agar plates or bright pinky-red colonies on the surface of MacConkey agar plates that are morphologically identical to the colonies of *E. coli*, the indole test would be performed as a confirmatory biochemical test. The syrup would pass the test if such colonies were not seen or if the confirmatory biochemical test was negative.

Detection of *Salmonella*

Salmonella is only investigated in herbal syrups according to ***European pharmacopoeia 2010*** with additional steps. 10 ml of each syrup was passed through a filter membrane that was then washed three times with sterile physiological serum. Each membrane was cultured in a tube containing sterile Brilliant Green Bile (BGB) broth as enrichment medium and the tubes were incubated for 48 h in 35 °C. Subsequently, by the culture loop after inflaming it, an extract was taken from each tube of a particular syrup and was spread on three XLD plates. The plates were incubated for 48 h at 35 °C, then visually observed to confirm that each syrup was free from *Salmonella* through the shape, specifications and color of the colonies after incubation.
If red colonies appeared on the surface of XLD plates and were morphologically identical to the colonies of *Salmonella*, we would move to reactions characteristic of Salmonella on Triple Sugar Iron (TSI) agar. If the former colonies belong to one of the *Salmonella* strains, colonies will appear red in color with black centers; at that point, a urease test would be performed as a confirmatory biochemical test. A negative urease test indicates the presence of *Salmonella*[27].
Table 3 shows the quantitative specifications of bacteria, fungi and organisms that must be absent in oral pharmaceutical preparations according to the *European Pharmacopoeia 2010.*

Table No. 3: Acceptance criteria of microbiological quality of non-sterile dosage forms (European pharmacopoeia 2010).

Route of administration	TAMC(CFU/mL)	TYMC(CFU/mL)	Specified micro-organisms
Aqueous preparations for oral use	10^2	10^1	Absence of *E.coli* (1 g/mL)
Herbal medicinal products to which boiling water is added before use	10^7	10^5	Not more than 10^3 *E coli* (1 g/mL) Absence of *Salmonella* (1 g/mL)

Karl Fischer Titration

The Karl Fischer titration was carried out with a KF titrator DL38 from Mettler-Toledo, Schwerzenbach, Switzerland using the two-component technique with Hydranal Titrant and Hydranal Solvent from Riedel- de Haën, Seelze, Germany. The polarising current for bivoltametric end-point determination was 10 µA and the stop voltage 100 mV; the drift was taken into account.

Halogen drying

The Moisture Analyser HR 73 from Mettler-To- ledo, Schwerzenbach, Switzerland was used. The sample is heated in an aluminium dish on the pan of the built-in balance by a circular halogen lamp. Additional glass-fibre pads can be used to obtain a more even distribution of samples. The maximum emission of the HR 73 is between 1 and 2 µm, it tends to shorter wavelengths with increasing temperature according to Wien's law. Optimal efficiency of such dryers is reached when the emission wavelengths correspond to the absorbance wavelengths of water in the range 1- 3.5 µm.

The halogen dryer used offers several drying pro- grammes. Within the programmes temperatures (in steps of 1°C from 50°C to 200°C) and stop criteria can be chosen. The drying process can be stopped manually, after a fixed time or at a chosen mass loss within a certain time.

Standard programme: The sample is heated to the set temperature as fast as possible possible and held constant at this temperature.

Rapid programme. The selected temperature is exceeded by 40% for 3 min to compensate for the cooling effect due to vaporisation and to accelerate the drying process. The temperature is then lowered to the chosen value.

Gentle drying. The temperature is continuously in- creased during a ramp time which can be freely chosen until the selected temperature is reached. Step drying. The drying is performed in three steps, each with a temperature and duration that can be freely chosen. In this investigation the standard, rapid and gentle drying programmes were used. 1 g of sample was ap- plied onto a glass-fibre pad by means of a 5-ml dispos- able syringe. The layer should be as thin and uniform as possible. The chosen stop criterion was a mass loss of 1 mg in 50 s in the standard and the gentle modes and 1 mg in 90 s in the rapid mode. The ramp time in the gentle program was 3 min.

RESULT AND DISCUSSION

Table No. 4: Phytochemicals screening test results for phytochemical constituents

Sr. no.	Phytochemical test	Preliminary test of *citrus medica linn.* Positive (+)/ Negative (-)	Preliminary test of *rubia cordifolia* Positive (+)/ Negative (-)	Preliminary test of *Bryophyllum pinnatum* Positive (+)/ Negative (-)
1.	Carbohydrate	+	+	+
2.	Alkaloids	+	+	+
3.	Protein	+	+	-
4.	Amino acid	+	+	-
5.	Steroids & Triterpenoide	+	-	+
6.	Saponin	-	+	+

7.	Tanin & phenolic	+	+	+
8.	Flavanoide	+	-	+

The preliminary phytochemical screening of Citrus medica Linn., Rubia cordifolia, and Bryophyllum pinnatum revealed the presence of various bioactive compounds. These results provide insights into the potential therapeutic properties of these plants and their suitability for use in formulations, particularly for the treatment of kidney stones.

Carbohydrates: All three plants tested positive for carbohydrates, indicating their potential as energy sources and their role in the stability and palatability of herbal formulations.

Alkaloids: The presence of alkaloids in all three plants suggests their potential pharmacological activities, including analgesic, anti-inflammatory, and antimicrobial effects. These properties are beneficial for treating kidney stones and associated symptoms.

Proteins: Both Citrus medica Linn. and Rubia cordifolia tested positive for proteins, while Bryophyllum pinnatum did not. Proteins can contribute to the overall nutritional value and may have specific therapeutic roles, such as enzyme inhibition in disease processes.

Amino Acids: Similar to proteins, amino acids were found in Citrus medica Linn. and Rubia cordifolia but were absent in Bryophyllum pinnatum. Amino acids play crucial roles in metabolic processes and could enhance the therapeutic efficacy of the formulations.

Steroids and Triterpenoids: Citrus medica Linn. and Bryophyllum pinnatum tested positive for steroids and triterpenoids, while Rubia cordifolia did not. These compounds have anti-inflammatory and analgesic properties, which are advantageous for managing pain and inflammation associated with kidney stones.

Saponins: Saponins were present in Rubia cordifolia and Bryophyllum pinnatum but absent in Citrus medica Linn. Saponins have diuretic properties, which can help in the prevention and dissolution of kidney stones by increasing urine flow.

Tannins and Phenolic Compounds: All three plants showed the presence of tannins and phenolic compounds. These compounds are known for their antioxidant properties, which help in reducing oxidative stress and protecting renal tissues from damage.

Flavonoids: Citrus medica Linn. and Bryophyllum pinnatum tested positive for flavonoids, while Rubia cordifolia did not. Flavonoids have significant antioxidant, anti-inflammatory, and diuretic effects, making them valuable for treating kidney stones.

The phytochemical profile of these plants indicates that they contain a range of bioactive compounds that could be beneficial in the treatment of nephrolithiasis. The presence of carbohydrates, alkaloids, tannins, and phenolic compounds across all three plants suggests a broad spectrum of therapeutic effects, including anti-inflammatory, analgesic, antioxidant, and antimicrobial activities. These properties are crucial in managing kidney stones and preventing their recurrence.

Citrus medica Linn., with its positive tests for carbohydrates, alkaloids, proteins, amino acids, steroids, tannins, phenolic compounds, and flavonoids, shows promise for its multifaceted therapeutic potential. Rubia cordifolia, despite lacking steroids, triterpenoids, and flavonoids, has saponins, which could enhance its diuretic effect. Bryophyllum pinnatum's profile, with the absence of proteins and amino acids but the presence of saponins, steroids, and flavonoids, indicates its complementary role in a multi-ingredient formulation.

The phytochemical analysis supports the use of these plants in herbal formulations for kidney stone treatment due to their diverse and synergistic bioactive compounds. Further research, including in-vivo studies and clinical trials, would be necessary to fully establish their efficacy and safety profiles.

Evaluation Parameters

Organoleptic Characteristics:

The evaluation of the organoleptic characteristics of the prepared herbal syrup provided the following observations:

Appearance: The formulation was found to be clear, indicating the absence of any particulate matter or turbidity, which is essential for patient acceptability and compliance.

Color: The syrup exhibited a brownish color, which is typical for herbal preparations and likely attributable to the natural color of the plant extracts used.

Odour: The formulation possessed a characteristic odour, which is expected from the herbal ingredients and is generally considered acceptable for medicinal syrups.

Taste: The syrup was orange-flavored, which enhances palatability, especially important for pediatric or sensitive patients.
pH: The pH of the syrup was measured at 6.5. Although slightly acidic, this pH is within a range that is generally safe for oral consumption and can potentially aid in the dissolution of kidney stones.
Microbial Limit: All herbal syrups were found to be free from *E. coli* and *Salmonella* as no descriptive colonies of the two microorganisms appeared in the specific media.
Viscosity: The viscosity of the syrup was found to be 17.479 cp (centipoise). This moderate viscosity is suitable for syrup formulations as it ensures ease of pouring and administration while providing a satisfactory mouthfeel.
Density: The density of the syrup was determined to be 2.152 gm/ml. This high density suggests a substantial concentration of dissolved components, which is typical for medicinal syrups and indicates that the formulation is adequately concentrated.
Specific Gravity: The specific gravity of the syrup was found to be 1.055. This value is slightly above that of water, as expected, due to the presence of sugars, plant extracts, and other excipients in the formulation. It confirms that the syrup is appropriately formulated to ensure stability and uniformity.
The **organoleptic properties** of the syrup, including its clear appearance, brownish color, characteristic odour, and orange flavor, make it an acceptable and palatable formulation for patients. The slightly acidic pH is advantageous for targeting kidney stone dissolution. The measured viscosity ensures that the syrup is easy to administer, while its density and specific gravity confirm the presence of an adequate concentration of active ingredients and excipients, ensuring therapeutic efficacy.
These results indicate that the herbal syrup formulation has been successfully prepared with desirable physical and sensory attributes. The data supports its potential use in the management and treatment of kidney stones, combining efficacy with patient acceptability. Further stability studies and clinical evaluations would be beneficial to confirm these findings and ensure long-term effectiveness and safety.

***In-vitro* study**
The study investigated the weight reduction of 7 mm and 10 mm kidney stones over a period of 48 hours using a specific herbal syrup formulation. The initial weight of the 7 mm kidney stone was recorded at 0.08 mg. After 18 hours, the weight decreased to 0.05 mg, showing a significant reduction. By the 24-hour mark, the weight further reduced to 0.04 mg and remained stable at this weight after 48 hours. Similarly, the 10 mm kidney stone initially weighed 0.21 mg. After 18 hours, the weight dropped to 0.15 mg, and after 24 hours, it further decreased to 0.13 mg. By the end of the 48-hour period, the weight of the 10 mm stone was 0.12 mg. These results indicate that the herbal syrup formulation effectively reduces the weight of kidney stones, with a significant initial reduction followed by a slower rate of dissolution. The data suggests that the formulation could potentially be effective in treating kidney stones, although further studies are recommended to confirm these findings and to optimize the formulation for improved efficacy.

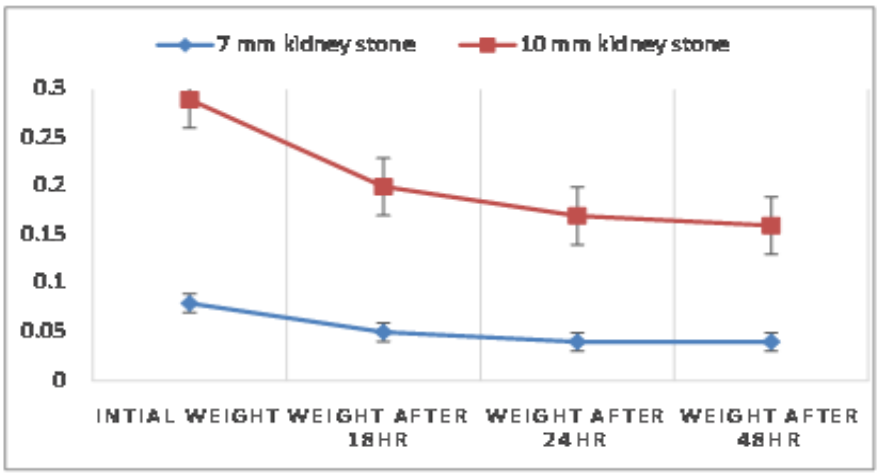

Figure 6 ***In-vitro* study**

$$a\%\ (10mm) = \frac{0.21 - 0.12}{0.21} \times 100 = 42.85\% \ldots(1)$$

$$A\%\ (7mm) = \frac{0.08 - 0.04}{0.08} \times 100 = 50\% \ldots(2)$$

Figure 5 7 mm stone initial weight

Figure 6 7 mm stone weight after 48 hrs.

Figure 7 10 mm stone initial weight

Figure 8 10 mm stone weight after 48 hrs.

Table 5: Stability Study of final batch

Time Duration	Temperature (°C) & Humididty	Appearance	Colour	Odour	pH	Microbial Limits
Initial (0)	40°C ± 2°C and a relative humidity (RH) of 75% ± 5%.	**NC**	**Brownish**	**Oragngy**	**6.5**	**10 CFU/mL**
After 1 Month	40°C ± 2°C and a relative humidity (RH) of 75% ± 5%.	**NC**	**NC**	**NC**	**NC**	**NC**
After 2 Month	40°C ± 2°C and a relative humidity (RH) of 75% ± 5%.	**NC**	**NC**	**NC**	**NC**	**NC**
After 3 Month	40°C ± 2°C and a relative humidity (RH) of 75% ± 5%.	**NC**	**NC**	**NC**	**NC**	**NC**
After 6 Month	40°C ± 2°C and a relative humidity (RH) of 75% ± 5%.	**NC**	**NC**	**NC**	**NC**	**NC**

Water Content by Karl Fischer titration

Water Content by Karl Fischer titration (10 replicates) and mass loss by halogen drying using different drying programmes at different temperatures (10 replicates each)**of Flavoured/Medicated syrup (Fructose) with relative standard deviation (rsd).**

Table 6: Water Content Determination of Medicated Syrup

Water Content 29.46% ±0.12%, rsd=0.42%, titration time 1-2 min.				
Programme	**Mass loss (%) at 100°C**	**Mass loss (%) at 105°C**	**Mass loss (%) at 110°C**	**Mass loss (%) at 115°C**
Gentle Drying	29.25± 0.14	29.46± 0.21	29.09± 0.17	29.32± 0.17
	rsd=0.48%	rsd=0.70%	rsd=0.58%	rsd=0.58%
	Time: 7-11 min.	Time: 7-8 min.	Time: 7-12 min.	Time: 7-11 min.
Standard Drying	26.06± 0.17	29.30± 0.14	29.45± 0.15	29.18± 0.12
	rsd= 0.58%	rsd= 0.84%	rsd= 0.49%	rsd=0.41%
	Time: 6-8 min.	Time: 6-8 min.	Time: 6-8min.	Time: 5-6 min.
Rapid Drying	29.36± 0.20	29.43± 0.17	29.46± 0.23	29.43± 0.15
	rsd=0.68%	rsd=0.58%	rsd=0.77%	Rsd=0.51%
	Time: 4-7 min.	Time: 4-7 min.	Time: 4-7 min.	Time 3-5 min.

CONCLUSION:

The herbal syrup was successfully formulated using *Citrus medica Linn., Rubia cordifolia*, and *Bryophyllum pinnatum* as key exipients. These botanicals were selected based on their traditional use and reported pharmacological properties for addressing renal calculi. Each exipients was thoroughly characterized for its phytochemical composition, which revealed the presence of active compounds such as flavonoids, alkaloids, saponins, and polyphenols. These compounds have been associated with diuretic, anti-inflammatory, and lithotriptic properties, essential for targeting renal calculi. The combination of *Citrus medica Linn., Rubia cordifolia,* and *Bryophyllum pinnatum* in the herbal syrup was designed to harness potential synergistic effects. Their combined action aims to dissolve stones, alleviate pain, and reduce inflammation associated with renal calculi. The herbal syrup underwent rigorous physicochemical characterization to ensure stability, viscosity, and palatability. Parameters such as appearance, colour, odour, pH, viscosity and density, microbial limit and water content were within acceptable ranges, indicating the formulation's quality and consistency. *In vitro* study. *In vitro* dissolution studies confirmed its ability to dissolve various types of renal calculi. The herbal syrup containing *Citrus medica Linn., Rubia cordifolia,* and *Bryophyllum pinnatum* presents a promising natural approach for the management of calcium oxalate renal calculi. Its formulation, characterized by potent phytochemicals and synergistic effects, offers a safe and effective alternative to synthetic

treatments. Further clinical trials are warranted to validate its therapeutic efficacy and establish its role in the clinical management of renal calculi.

CONFLICT OF INTEREST: The authors have no conflicts of interest regarding this investigation.

ACKNOWLEDGEMENT: The authors express their sincere thanks to Dean, Teaching and non-teaching staff, Dr. Subhash University, Junagadh, Gujarat, India for providing guidance and support to this research work.

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