



Evaluation Of Synergistic Amalgamation of Medicinal Herbs In the Treatment of Urolithiasis

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ABSTRACT

Urolithiasis commonly referred to as kidney stones occurs due to the intricate connection of dietary practices, environmental factors, genetics and urinary tract anatomy which varies substantially with prevalence rising globally as a consequence of lifestyle changes. Ayurvedic medicinal herbs offer a natural and holistic approach in managing urolithiasis, minimizing recurrence and side effects, compared to allopathic treatments, which often carry risks like gastrointestinal disturbances and renal toxicity. In this regard a unique blend of three medicinal herbs- *Aerva lanata*, *Tribulus terrestris*, and *Eugenia jambolana* are combined to improve the therapeutic effectiveness which showed amalgamation revealing phytoconstituents that had stone dissolving properties confirmed through FTIR studies. This herbal blend in liquid form is a novel approach to treat calculi that occur in the urinary tract with genuine attempt being made to create a novel liquid formulation which achieved a synergistic effect that showed the removal of calculi from the urinary system yet retaining safety. The synergistic effect of liquid formulation was studied by taking male albino rats and administering 0.75 % v/v of Ethylene glycol mixed with purified water to induce urolithiasis. The administration of the liquid formulation was given from day 15-28 and the analysis of biochemical parameters like oxalate, calcium and phosphate from urine samples and calcium, uric acid and creatinine from serum were analyzed. The study revealed the anti-urolithiatic activity of the liquid formulation through inhibition of biochemical parameters which supports treatment of urolithiasis.

Keywords: Urolithiasis, *Aerva lanata*, *Tribulus terrestris*, *Eugenia jambolana*, FTIR spectrum, Phytoconstituents.

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Received 25 August 2025, Accepted 12 September 2025

Please cite this article as: Shankar KR *et al.*, Evaluation Of Synergistic Amalgamation of Medicinal Herbs In the Treatment of Urolithiasis. American Journal of Pharmacy & Health Research 2025.

INTRODUCTION

Urolithiasis is a significant global health concern, marked by the formation of kidney stones, recurring pain, hematuria and shows potential complications like infections or renal damage affecting millions globally [1]. Its management often involves a combination of dietary changes, medications or surgical interventions. Allopathic treatments despite providing rapid symptom relief, they are often associated with significant side effects and fail to address the root cause of the condition [2-4]. In contrast, Ayurveda, an ancient system of medicine, offers a holistic and sustainable approach, emphasizing natural remedies to manage and prevent urolithiasis effectively. Ayurvedic medicinal herbs with proven efficacy, minimal side effects and preventive benefits, might represent a promising solution in treating urolithiasis [5-7]. Hence, in this aspect the researchers made an attempt to scrutinize the combined effects of medicinal herbs which might show synergistic effect and could be favourable in dissolving the stones. In this consequence 3 medicinal herbs- *Aerva lanata*, *Tribulus terrestris* and *Eugenia jambolana* were taken up for the study.

Aerva lanata, commonly known as Mountain Knot Grass has anti-lithogenic property which helps dissolve and prevent stone formation by reducing oxalate and calcium aggregation and also prevents oxidative damage to kidney tissue due to its anti-oxidant activity that scavenges free radicals [8-10]. *Tribulus terrestris*, a member of *Zygophyllaceae* Family commonly known as Puncture vine (or) Gokshur inhibits stone formation by reducing the crystallization of calcium and oxalates and also promotes urination to flush out small crystals. It also complements anti-oxidant effect by stabilising cell membrane against oxidative stress [11-14]. Similarly, *Eugenia jambolana* of *Myrtaceae* Family, commonly known as Java Plum demonstrates anti-oxidant activity that can mitigate oxidative stress which is a key factor in kidney stone pathogenesis [15]. Individually, each of these medicinal plants possesses pharmacological properties that target different mechanisms involved in the formation and management of urolithiasis which by combining may enhance the efficacy through complementary actions.

MATERIALS AND METHOD

Chemicals and Equipment:

All the chemicals used are of analytical grade.

Estimation of biochemical parameters done by using Biochemical analyzer of Robonic India Pvt. Ltd.

Preparation of Liquid Dosage Form:

The 3 medicinal plant (*Aerva lanata*, *Tribulus terrestris* and *Eugenia jambolana*) powders were procured, sieved and mixed uniformly. 2 grams of test powder was collected and decoction is prepared by using 100 ml of purified water for 15-30 mins by the application of heat. From the above mixture 90 ml of liquid sample or decoction was cumulated and Carbopol 934, polysorbate-80 were added by uniform mixing which acts as emulsifiers, stabilizers and solubilizers. To this mixture excipients like sodium benzoate, vanillin was added for their preservative and flavouring property and also Stevia was incorporated into the solution as a natural sweetener. The pH was adjusted between 6.5-7.5 by using sodium citrate and made up to 50 ml using purified water as discussed in table-1.

Table 1: Ingredients of Liquid formulation.

S.No	Ingredients	Weight
1.	Mixture of 3 powders	2 g
2.	Carbopol 934	0.5 g
3.	Polysorbate-80	0.3 g
4.	Sodium benzoate	0.1 g
5.	Sodium citrate	0.1 g
6.	Vanilla	0.1 g
7.	Stevia	0.1 g
8.	Purified Water	Quantity sufficient
Total		50 ml

Animals used:

Male Albino rats weighing 200-250 grams were selected for the study. Animals were acclimatized to the laboratory under control conditions 7 days before dosing. Total 20 animals were taken for the study and were divided into 4 groups, each having 5 rats.

Induction of Urolithiasis ¹⁶⁻¹⁹

To induce urolithiasis to the experimental animals, 0.75 % v/v of Ethylene Glycol mixed with purified drinking water was administered to all the group of animals for a period of 28 days. The administration of liquid formulation and standard drug Cystone were started from day 15.

Grouping of Animals:

Group-I rats served as Control that received normal food with purified drinking water.

Group-II animals were given 0.75% v/v of Ethylene Glycol mixed with purified drinking water for induction of Renal stones up to 28 days.

Group-III rats were administered with 1ml of liquid formulation which received Ethylene glycol from day 15 till 28 days.

Group-IV animals received standard drug Cystone (100 mg/kg) from day 15-28.

Collection of Urine Samples ^{[20]:}

Rats were taken from metabolic cages and the urine samples were collected on 28th Day for up to 24 hours. Concentrated Hydrochloride was added to the urine sample before its storage at 4°C.

Assessment of Oxalate:

To 1ml of Urine sample, 2-3 ml of 2N Sulfuric acid was added and made up to 10ml by the addition of purified water. The preparation was heated on a water bath at 70-80 °C and later titrated with 0.01 N Potassium permanganate till it produced pale pink colour as end point.

Collection of Serum Sample^[21]:

Blood was collected from the tail vein of the animal at the end of the experiment as the serum was separated by centrifugation at 3000 RPM.

FTIR Studies:

The sample was subjected to FTIR studies which showed peaks from 625 cm⁻¹ to 3000 cm⁻¹ as shown in figure-1.

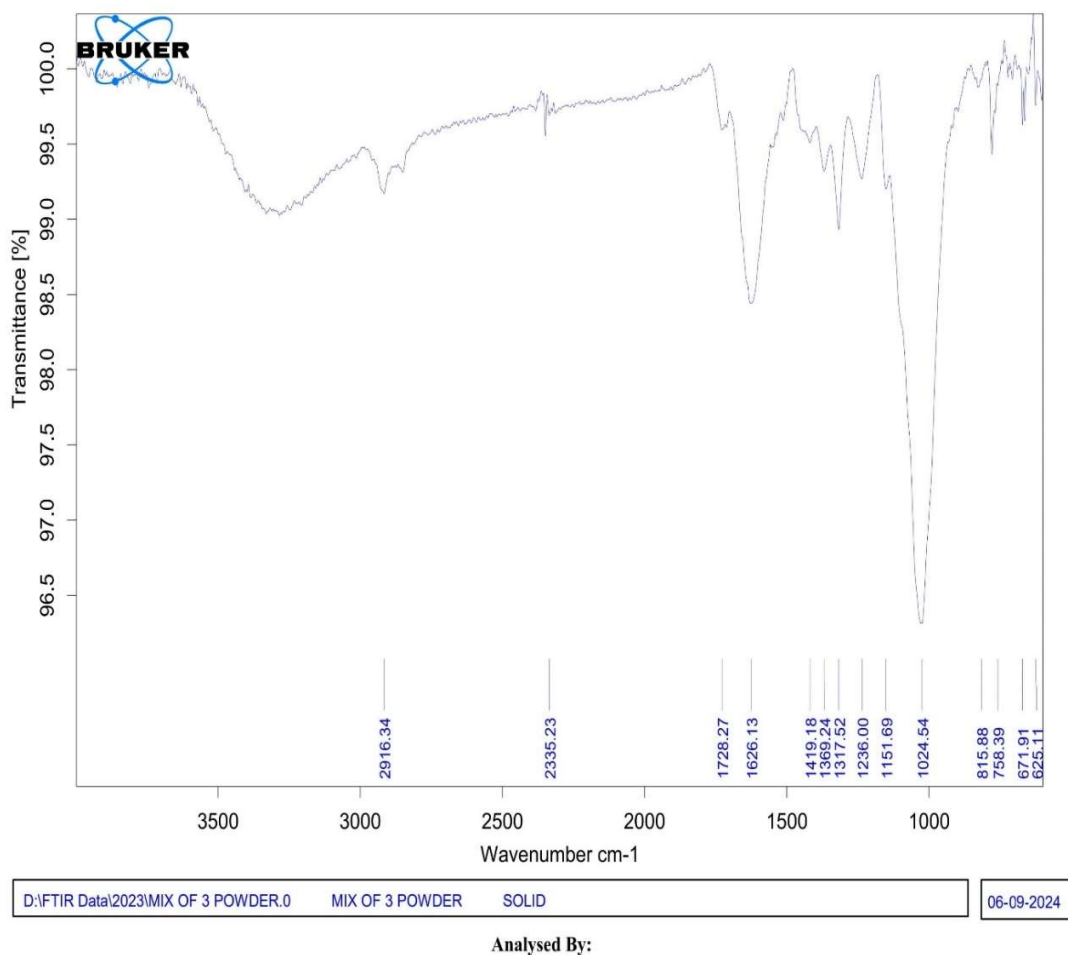


Figure 1: FTIR Spectrum of the Liquid Formulation.

FTIR studies related to amalgamation of these three medicinal herbs currently used for prevention and treatment of kidney stones indicated the presence of Phenolic, Flavonoid compounds and Terpenoids which may contribute to anti-oxidant, diuretic and stone dissolving properties. The presence of these active compounds may help to break down the stone forming process and prevent new stone formation, the presence of flavonoids, saponins, polyphenols have well documented activities that can aid in flushing of kidney pebbles.

RESULTS AND DISCUSSION

***In-vitro* Evaluation Parameters of Liquid Dosage Formulation:**

For the even dispersion of particles and to prevent the settling of particles at the bottom of the container, the liquid dosage form was prepared using 3 different types of suspending agents at different ratio 0.1, 0.3, 0.9g of Gellan gum (Natural), Hydroxy propyl methyl cellulose K 100 (Semi synthetic) and Carbopol 934 (Synthetic) as shown in table-1.

Compositions used are C1, C2, C3 are Gellan Gum

C4, C5, C6 are HPMC K 100

C7, C8 and C9 are Carbopol 934

where Carbopol [C7, C8 and C9] gave the best result.

pH of all formulation was found to be in the range of 7.01 – 7.31.

Increase in **temperature** reduces the viscosity for all formulation

Drug content for all batches was found to be in the range of 92- 99%

Dissolution studies showed the % of drug release mentioned in table-3

Table 2: Assessment of Different Compositions of Liquid Dosage Form

Parameter	Batch Code								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
Degree of flocculation	1.19±1.2	2.21±6.3	2.57±1.1	2.99±0.1	3.10±0.2	3.31±0.3	3.55±0.1	3.80±0.3	4.01±
	3	4	2	8	6	2	9	8	0.18
No of shaking for complete dispersion	03	04	06	04	06	07	09	11	12
Drug content	92.65±0.	94.85±0.	95.09±0.	95.96±0.	97.8±0.0	98.36±0.	96.57±0.	97.97±0.	99.12
	08	15	09	15	9	18	24	19	±0.33
Viscosity	0.29	0.35	0.48	0.35	0.56	0.69	0.55	1.56	2.28

Table 3: Dissolution studies of Liquid Dosage Formulation

Time (mins)	Drug release (%)
5	30.14±4.41
10	40.70±5.21
15	49.81±5.63
30	56.56±2.36
45	75.53±4.40
60	94.65±1.53

***In-vivo* Evaluation Parameters of Liquid Dosage Formulation**

Administration of Ethylene glycol induced Urolithiasis to the experimental animals when mixed with drinking water for a period of 28 days produced renal calculi in all groups. Different biochemical parameters of the experimental animals were collected and analyzed from both urine as well as serum samples which was detailed in table-4,5 and represented in figure-2,3.

Table 4: Assessment of Urine Biochemical Parameters of Anti-Urolithiatic Activity of Liquid Formulation:

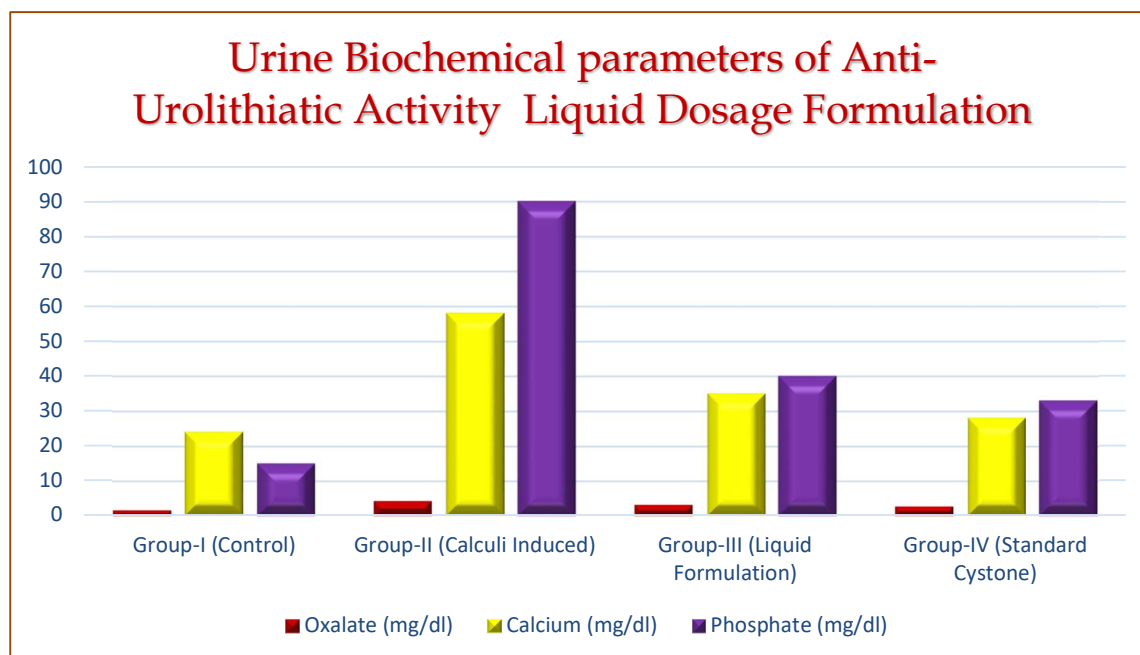
Urine Parameters (mg/dl)	Group-I (Control)	Group-II (Calculi Induced)	Group-III (Liquid Formulation)	Group-IV (Standard Cystone)
Oxalate (mg/dl)	1.6±0.14	4.2±0.45	3.1±0.03	2.7±0.19
Calcium (mg/dl)	24±0.08	580±0.20	35±0.14	28±0.13
Phosphate(mg/dl)	15±0.21	90±0.04	40±0.62	33±0.28

Values are expressed as mean ± SEM P ≤ 0.001

Table-5 Assessment of Serum Biochemical Parameters of Anti-Urolithiatic Activity of Liquid Formulation:

S.NO	Creatinine (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)
Group-I (Control)	1±0.25	6.5±0.26	9.3±0.19
Group-II (Calculi Induced)	2.8±0.53	12±0.24	11.5±0.62
Group-III (Liquid Formulation)	2±0.0.2	7.8±0.13	10.7±0.40
Group-IV (Standard Cystone)	1.4±0.18	7.4±0.21	9±0.10

Values are expressed as mean ± SEM P ≤ 0.001



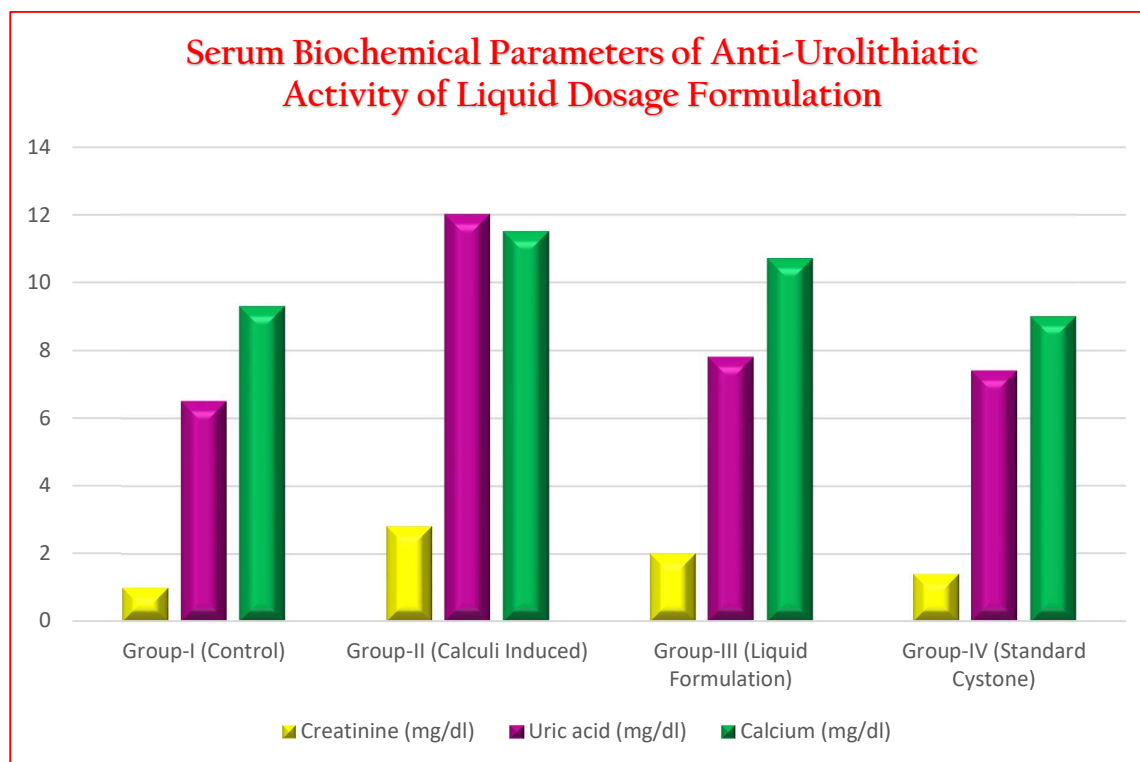


Figure 3: Assessment of Serum Biochemical Parameters of Anti-Urolithiatic Activity of Liquid Formulation:

DISCUSSION

Urolithiasis or stone formation in the urinary system is considered to be a complex condition caused due to the formation of calculus which is occurred because of imbalance between inhibitors and promoters in the kidneys. Saturation of urine is considered as the starting process for the formation of stones ^[1] which leads to crystallization and aggregation of calcium, uric acid, oxalates and phosphates etc.,

Despite the marketed allopathic formulations available in treating urolithiasis, it always has a backdrop of adverse reactions questioning the safety of medications. In the recent era, the world is mostly looking around for the Ayurvedic formulations due to its less notable side effects and safety margin. In this study, we tried to highlight the action of Ayurvedic medicinal herbs by combining 3 medicinal herbs- *Aerva lanata*, *Tribulus terrestris* and *Eugenia jambolana* with already-known stone reduction activity, so that a synergistic action could be rendered. Hence, in this study, despite using the normal extracted medicinal herbs we prepared a liquid formulation combining these 3 plants and adding different ingredients to highlight the test liquid formulation. The formulated liquid dosage form with the blend of medicinal herbs was superior to many

Ayurvedic formulations as our indigenous preparation possessed robust scientific approach focused on efficacy, safety and stability.

In the research study, 4 group of animals were taken and were subjected to induction of urolithiasis till 28 days, where the group which received formulated liquid dosage reduced the oxalate, calcium and phosphate levels in the urine and simultaneously when tested the serum samples the biochemical parameters like calcium, creatinine and uric acid were also notably reduced in comparison to the standard drug Cystone.

The developed formulation reduced stone formations and ensured fast absorption, bioavailability, shelf-life and storage safety.

CONCLUSION

The indigenous liquid formulation amalgamated with 3 medicinal herbs used for the prevention and treatment of kidney stones is an innovative design dosage form which reduced serum creatinine, uric acid and calcium concentration and also markedly reduced the presence of oxalates, phosphates and calcium in urine samples. This research work is the new developmental combination of medicinal herbs for the effective treatment of urolithiasis.

ACKNOWLEDGEMENT

The authors are thankful to the administration of Aditya College of Pharmacy for providing the required facilities for conducting the research work.

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