



Original Research Article

Phytochemical analysis and *in vitro* evaluation of antiurolithiatic potential of *Jasminum officinale* rootS. Shervin Jose^{1*} ¹Dept. of Pharmacology, S.A. Raja Pharmacy College, Tirunelveli, Tamil Nadu, India.

Abstract

Background: Currently, there is no published scientific evidence regarding the *in vitro* antiurolithiatic properties of *Jasminum officinale* root. Therefore, this study represents the first attempt to evaluate the antiurolithiatic activity of the root of *Jasminum officinale*.

Objectives: The aim of this study was to investigate the *In vitro* antiurolithiatic activity and preliminary phytochemical screening of the chloroform and ethanolic extracts of *Jasminum officinale* root.

Methods: Fresh roots of *Jasminum officinale* were extracted in ethanol and chloroform and arranged in varying quantities. Also studied were the Nucleation, Growth, and Aggregation assays, and the extract was compared to the standard drug Cystone.

Result: Based on the results of the anti-urolithiatic action nucleation, growth, and aggregation assays, the ethanolic extract at 1000 µg/ml demonstrated a greater percentage of inhibition than the standard drug, Cystone.

Conclusion: The root of *Jasminum officinale* exhibits inhibitory activity on calcium oxalate (CaOx) crystallization, which suggests that its ethanolic and chloroform extracts can have therapeutic significance in the treatment of urolithiasis. These findings justify the conventional use of this plant for treating urinary stone diseases. Nonetheless, to ascertain the clinical utility and safety of *Jasminum officinale* as an antiurolithiatic drug, additional intensive investigations involving well-designed preclinical experiments and controlled clinical trials are crucial.

Keywords: *Jasminum officinale*, Antiurolithiatic, Cystone, Nucleation assay, Growth assay

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1. Introduction

It is a widespread issue that can affect people of all ages and is a leading contributor to morbidity globally. Patients between the ages of 20 and 60 years are typically diagnosed with a primary illness. If left untreated, urolithiasis can naturally progress to consequences, such as kidney loss, urinary tract infections, and urinary tract abnormalities. According to estimates, the recurrence rate of secondary stone development is 10–23% annually, 50% every 5–10 years, and 75% every 20 years for patients.

Primarily, four types of stones have been identified: calcium, uric acid, struvite, and cystine. Calcium oxalate (CaOx) constitutes the bulk of stones.¹ Urine supersaturation causes precipitation and crystallization of minerals that cause kidney stones, which results in urolithiasis. Calcium oxalate

is the most common mineral causing stone formation. Urolithiasis has been treated using herbal medicines in Ayurveda.²

Traditional medical practices, particularly the use of medicinal plants, are essential for fulfilling the basic health needs of underdeveloped countries. In recent decades, the use of herbal treatments has increased in wealthy countries. Plants are a major source of medicinal compounds. The active ingredients of many medications are found in plants or generated as secondary metabolites. The remarkable contribution of plants to the drug industry was possible because of the enormous number of physicochemical and biological investigations conducted worldwide. The plant *Jasminum officinale* Linn., a member of the family Oleaceae, is a small shrub native to warm Asia and is grown there. Its chemical constituents include salicylic acid, resin, indole,

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alcohol, and jasmine (alkaloid). Its traditional uses include analgesic, antidepressant, anti-inflammatory, antiseptic, aphrodisiac, sedative, expectorant, and tonic (uterine) effects. *Jasminum officinale* leaves showed allelopathic activity, and spectral data identified the primary active ingredient as oleuropein, a secoiridoid glucoside.

The plant's flowers were found to have pharmacological uses as sedatives, tonics, astringents, and anaesthetics, as well as central nervous system depressant activity.³⁻⁶

Jasmine is used to treat stress, depression, and certain respiratory disorders. Its aroma is said to be calming and soothing without being soporific. Additionally, it is recommended for sensitive skin disorders. However, jasmine is primarily used to treat a variety of sexual issues and has a reputation as an aphrodisiac.⁷⁻⁸

Indians frequently use *Jasminum* to treat skin conditions; the leaf juices can be used to clear up corns and treat mouth ulcerations; the anti-secretory and anti-oxidant components of *Jasminum* may also treat peptic ulcers; additionally, the ethanolic extract of *Jasminum* produces an antibiotic effect upon typhoid fever and staph infections; they emphasized that Jasmine oil may serve as a mainstream antibiotic treatment; the leaf juice is applied to corns and ear discharges; the barks and leaves contain salicylic acid and are used as analgesics, fibrifuge, etc.; the root is used to treat ringworm; and the flowers are aphrodisiac, antiseptic, antispasmodic, and tonic.⁹⁻¹⁰

Among its applications in urinary tract infections and as a diuretic, *J. officinale*'s stem, bark, and root leaves have shown considerable antibacterial action against reference bacteria.¹⁰

Alkaloids, coumarin, emodin, flavonoids, phenol, saponins, sesquiterpenoids, secoiridoids, and tannins are some of the main phytochemicals found in *Jasminum officinale*. These compounds are known to have specific pharmacological activities that give them their therapeutic qualities.¹¹

Using nucleation, growth, and aggregation assays, the current study examined the impact of chloroform and ethanol extracts of *Jasminum officinale* root on in-vitro urolithiasis by utilizing its anti-crystallization properties.

2. Materials and Methods

2.1. Collection of plant material and authentication

In April, the Root of *Jasminum officinale* was collected from Marthandam, Kanyakumari district and authenticated by Dr. S. Jaya kumar, associate professor of botany N.M.Christian College, Marthandam, Tamil Nadu.

2.2. Preparation of extract

After being allowed to dry in the shade at room temperature, the plant root was ground into a coarse powder using a dry grinder. A Soxhlet device containing 100 grams of this coarse powder was used to extract it in stages using 500 milliliters of ethanol and chloroform. The extraction process was repeated until the solvent's color in the siphon tube turned colorless. Ethanol and chloroform extracts were evaporated at 60°C using a rotary evaporator.

2.3. Phytochemical study

2.3.1. Qualitative screening

The Chloroform and ethanolic extracts of the roots of *Jasminum officinale* were screened for the phytochemical constituents according to the standard methods^{Error! Reference source not found.-13} The results are given in Table

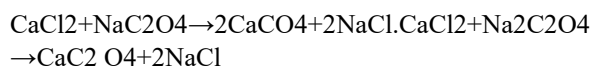
2.4. In vitro antiurolithiatic activity

2.4.1. Nucleation assay method

Its simplicity and good reproducibility make it the traditional model for studying oxalate crystallization. This methodology evaluates the inhibitory ability of any chemical species by studying crystallization both with and without an inhibitor. In a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5, 950 µL of calcium chloride solution was combined with 100 µL of herb extracts at various concentrations (100 µg/ml 1000 µg/ml) to create solutions of calcium chloride and sodium oxalate at final concentrations of 5 mmol/L and 7.5 mmol/L, respectively. 950 µL of sodium oxalate solution was added to initiate crystallization.

A constant temperature of 37 °C was maintained. At 620 nm, the solution's OD was observed. By contrasting the induction time with the control in the presence of the extract, the rate of nucleation was calculated. The Cystone tablets serve as a standard solution.^{Error! Reference source not found.}

Crystal growth was anticipated as a result of the subsequent reaction:



2.5. Growth assay

Calculus is a tiny hard mass that can arise from the combination of recently created crystals. Both with and without medication extracts, the percentage suppression of calcium oxalate crystal formation was assessed. A 1.5 ml solution of sodium chloride (10 mM) buffered with Tris (10 mM) at pH 7.2 was mixed with 1 ml of each of 4 mM calcium chloride and 4 mM sodium oxalate. A crystal slurry of calcium oxalate monohydrate (1.5 mg/ml acetate buffer) was added in 30 µl. Oxalate consumption starts as soon as calcium oxalate monohydrate crystal slurry is added, and absorbance at 214 nm was observed for 600 seconds, both with and

without extract. The standard medication solution is Cystone tablets.

The relative inhibitory activity was calculated as follows: % relative inhibitory activity = $((C-S)/C) \times 100$.

Where "C" represents the rate of free oxalate reduction in the absence of any extract and "S" represents the rate of free oxalate reduction in the presence of drug extract.¹³⁻¹⁵

2.6. Aggregation assay

Calcium chloride and sodium oxalate were combined at a concentration of 50 mmol/L to create CaOx monohydrate (COM) crystals. After one hour of equilibration to 60 °C in a water bath, both solutions were overnight cooled to 37 °C. Centrifugation was used to collect the crystals, which were subsequently evaporated at 37 °C. At a final concentration of 0.8 mg/ml, CaOX crystals were employed, buffered with 0.05 mol/L Tris and 0.15 mol/L NaCl at pH 6.5. After ceasing the stirring, experiments were carried out at 37 °C with or without the plant extract. Cystone pills serve as a standard medication solution.¹⁶⁻¹⁸

Next, using the following calculation, the turbidity in the extract-containing solution was compared to that in the control to determine the percentage aggregation inhibition rate (Ir):

$$Ir = (1 - \text{Turbidity}_{\text{sample}} / \text{Turbidity}_{\text{control}}) \times 100.$$

3. Results

3.1. Qualitative screening

The phytoconstituents present in chloroform extracts of such as Alkaloids, Carbohydrates, Saponins, Phenolic compounds, Flavonoids, and Terpenoids. Ethanolic extract showed the presence of Alkaloids, Glycosides, Saponins, Phenolic compounds, Terpenoids, and Steroids. (Table 1)

Table 1: Qualitative phytochemical screening of *Jasminum officinale* root extract of chloroform and ethanol

Constituents	Cerjo	Eerjo
Alkaloids	Present	Present
Carbohydrate	Present	Absent
Glycosides	Absent	Present
Saponins	Present	Present
Protein and Aminoacids	Absent	Absent
Phenolic compound	Present	Present
Terpenoids	Absent	Present
Steroids	Absent	Present
Aminoacids	Absent	Absent
Flavanoids	Present	Absent

Cerjo – Chloroform extract of root of *Jasminum officinale*

Eerjo – Ethanolic extract of root of *Jasminum officinale*

3.2. Nucleation assay

The crystallization of calcium oxalate particles in the urinary system is responsible for urine supersaturation. With the addition of fresh components, the salts that form stones start to join into clusters during this nucleation phase. When it came to the nucleation of calcium oxalate salts, the ethanolic extract and chloroform of *Jasminum officinale* showed greater inhibitory action than the Cystone standard solution.

Since nucleation is a crucial first step for the start of crystals, which subsequently develop and form aggregates, an in vitro crystallization study was conducted. By preventing calcium oxalate from nucleating and dissolving into smaller particles as the fraction's concentration increased, extracts of *Jasminum officinale* prevented crystallization. Both extracts exhibit action that is dependent on dosage. (Table 2, Figure 1)

Table 2: Antiurolithiatic effect of various chloroform and ethanolic extract concentrations of *Jasminum officinale* root using the nucleation assay method

S. No	Concentration (µg/ml)	CERJO	EERJO	Standard drug
1.	200µg/ml	58.4%±0.0018**	60.1%±0.0020**	80.11%±0.0011*
2.	400µg/ml	71.20%±0.0012*	74.50%±0.0012**	79.23%±0.0024*
3.	600µg/ml	79.24%±0.054*	80.23%±0.078**	81.60%±0.0036*
4.	800µg/ml	85.26%±0.0023**	87.2%±0.0012**	85.20%±0.004*
5.	1000µg/ml	92.2%±0.0034**	93.4%±0.005**	91.3%±0.0021**

Mean ± SEM, n = 6 the values are expressed. When compared to the standard, the values are significant, with * p<0.05 and **p<0.01

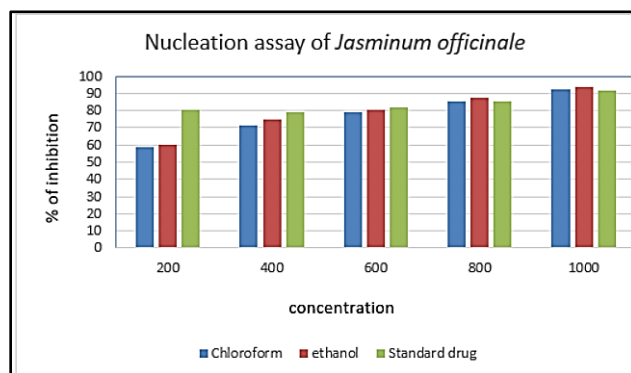


Figure 1: Nucleation assay on the root extract of *Jasminum Officinale*

4. Growth Assay Method

In comparison to regular Cystone tablets, the findings of the growth assay for anti-urolithiatic activity showed that the

ethanolic extract at 1000µg/ml had a higher percentage of inhibition. (Table 3, Figure 2)

Table 3: Anti-urolithiatic effect of various chloroform and ethanolic extract concentrations of *jasminum officinale* root using the growth assay method

S. No	Concentration (µg/ml)	CERJO	EERJO	Standard drug
1.	200µg/ml	58.4%±0.0018**	60.1%±0.0020**	80.11%±0.0011*
2.	400µg/ml	71.20%±0.0012*	74.50%±0.0012**	79.23%±0.0024*
3.	600µg/ml	79.24%±0.054*	80.23%±0.078**	81.60%±0.0036*
4.	800µg/ml	85.26%±0.0023**	87.2%±0.0012**	85.20%±0.004*
5.	1000µg/ml	92.2%±0.0034**	93.4%±0.005**	91.3%±0.0021**

Mean ± SEM, n = 6 is the way the values are expressed. Compared to the standard, the values are significant: * p<0.05; ** p<0.01.

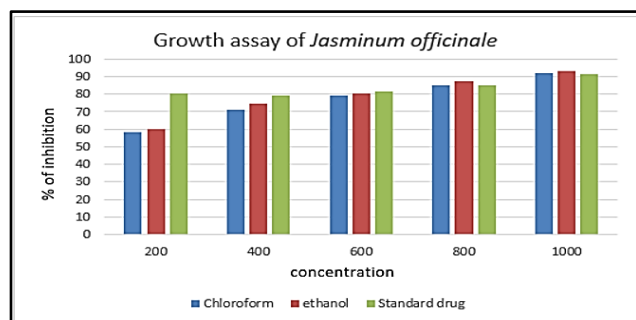


Figure 2: Growth assay on the root extract of *jasminum officinale*

Table 4: Anti-urolithiatic effect of various chloroform and ethanolic extract concentrations of *jasminum officinale* root using the aggregation activity

S. No	Concentration (µg/ml)	CERJO	EERJO	Standard drug
1.	200µg/ml	63.1%±0.0021**	65.4%±0.0007* *	64.64%±0.011*
2.	400µg/ml	73.5%±0.080**	75.60%±0.036*	74.11%±0.028*
3.	600µg/ml	82.2%±0.070**	84.20%±0.040*	78.24%±0.012*
4.	800µg/ml	90.2%±0.070**	91.17%±0.075* *	92.1%±0.036**
5.	1000µg/ml	93.2%±0.022**	97.14%±0.070* *	95.6%±0.043**

Mean ± SEM, n = 6 is the way the values are presented. When compared to the standard, the values are significant, with * p<0.05 and ** p<0.01.

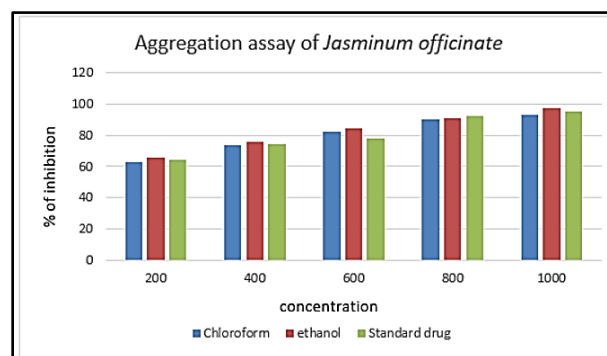


Figure 3: Aggregation assay on the root extract of *Jasminum Officinale*

4.1. Aggregation assay

Crystals of calcium oxalate start to form, group together with other crystals, and are eventually retained in the kidney. This process of accumulation results in kidney damage. When compared to Cystone standard solution, the ethanolic extract of *Jasminum officinale* produced marginally better results in aggregation assay. (Table 4, Figure 3)

5. Discussion

According to the results of the nucleation assay for anti-urolithiatic action, the ethanolic extract at 1000 µg/ml showed a higher percentage of inhibition than the standard medication (cystone tablets). The anti-urolithiatic activity, as determined by growth test results, showed that the ethanolic extract at 1000 µg/ml had a higher percentage of inhibition than regular cystone tablets. The aggregate assay findings for anti-urolithiatic action showed that the percentage inhibition of the ethanolic extract was higher than that of the standard medication cystone tablets. Because plant roots contain abundant amounts of alkaloids, saponins, phenolic compounds, and flavonoids, they demonstrate strong antilithiatic efficacy. One-way analysis of variance was used for statistical analysis.

6. Conclusion

The current research proves that the root extracts of *Jasminum officinale*, especially the chloroform and ethanolic fractions, have a remarkable inhibitory action on calcium oxalate (CaOx) crystallization, a key pathological process in the formation of urolithiasis. These results indicate the possible efficacy of these extracts as antiurolithiatic agents.

However, despite the encouraging in vitro results, more extensive full-scale investigations must be conducted to validate these early findings. Exhaustive preclinical studies using adequate in vivo models, followed by properly designed clinical trials, should be conducted to assess the effectiveness, safety, pharmacokinetics, and mechanism of action of such plant-derived materials. Such proof is necessary to justify their therapeutic use for the prevention and treatment of urolithiasis.

Currently, the cost of treating urolithiasis is very high, and the medications used to treat it have negative side effects. Invasive kidney stone treatment techniques can put significant strain on the healthcare system and result in major problems. Many diseases can be managed and treated as alternatives to traditional remedies. Unlike contemporary medications, traditional herbal remedies are efficient and rarely cause adverse side effects.

Numerous herbal remedies have been used to treat urolithiasis and to lower the risk of kidney stone recurrence. However, we do not fully understand how phytochemicals function. Through changes in urine composition, such as lowering calcium and raising magnesium concentrations, facilitating stone passage by increasing urine volume, enhancing renal function, controlling oxalate metabolism, preventing the crystallization process, and raising antioxidant levels in renal tissues, the herbal medications demonstrate their antiurolithiatic properties.^{19–21}

7. Acknowledgement

Department of Pharmacology S.A. Raja College of Pharmacy, Tirunelveli, Tamilnadu.

8. Source of Funding

None.

9. Conflict of Interest

None.

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