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In Vitro Investigation of Anti-Urolithiatic Potential from Different Types of Hydroalcoholic Plant Extracts

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Abstract: Kidney stones, or urolithiasis, are a worldwide medical challenge to the urinary system. Pentacyclic triterpenoids occur in nature as the main constituents of several plants and are reported to have an excellent protective effect against kidney stones. The current *in vitro* investigation aims to compare the effects of hydroalcoholic extracts of the bark of *Madhuka longifolia* (Sapotaceae) (sample 1), the leaves of *Catharanthus roseus* (Apocynaceae) (sample 2) and *Salvia officinalis* (Lamiaceae) (sample 3) on calcium oxalate kidney stones. The anti-urolithiatic impact of plant extracts (Samples 1, 2, and 3) was assessed in the current study utilising the nucleation and aggregation method at increasing concentrations (100, 250, 500, 750, and 1000 µg/ml). After the initial screening for phytochemicals, terpenoids were found in all of the extracts, indicating their presence. The crystallisation of calcium oxalate was caused by calcium chloride and sodium oxalate. The results of the nucleation and aggregation assay from all samples are significant as compared to the control. In nucleation assays, sample 1 showed significant results as compared to samples 2 and 3. Sample 1 showed a maximum percentage inhibition of 77.42% at 1000 µg/ml concentration, and its 50% inhibitory concentration was calculated as 54.49 µg/ml. In an aggregation assay among three samples, sample 2 showed significant results. The percentage inhibition of sample 2 was 80.87 % at 1000 µg/ml and its 50% inhibitory concentration value was calculated as 57.90 µg/ml. As a result, the current study supports the finding of the most effective hydroalcoholic extract derived from the above-mentioned plants against anti-urolithiatic efficacy, providing a pathway for further isolating the lead bioactive compounds responsible for the anti-urolithiatic effect.

Keywords: Anti-urolithiatic activity, Crystallisation, Nucleation assay, Aggregation assay, Kidney stones

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Introduction

Urolithiasis, characterized by the formation of kidney stones within the urinary tract, is a globally

prevalent and debilitating condition. It is associated with symptoms such as colicky

discomfort, vomiting, dysuria, haematuria, pyuria, and oliguria (Santhoshi *et al.*, 2015; Madan and Ahmad, 2018). The formation of kidney stones is influenced by two key factors: the supersaturation of urine and an imbalance between substances that promote and inhibit crystal formation. During the initial stage of nucleation, the smallest crystal units, known as "nuclei" or "nidus," are formed. Subsequently, these calcium oxalate nuclei undergo aggregation, leading to the formation of larger particles. Hence, preventing crystal retention, nucleation, and aggregation represents a potential approach to reduce stone formation. Surgical removal of stones is an essential component of modern urolithiasis treatment. However, techniques like extracorporeal shock wave lithotripsy (ESWL) and pharmacological interventions such as diuretics do not guarantee the prevention of stone recurrence (Alealign and Petros, 2018). Moreover, recent studies have highlighted the potential risks associated with therapeutic doses of shock waves, including kidney damage, dysfunction, and an increased recurrence rate of stones. Furthermore, these treatments have been accompanied by rising costs (Selvam *et al.*, 2001).

Considerable advancements have been made in the field of medicine; however, the complete cure of kidney stones still remains elusive through existing medications or therapies. Therefore, there is a pressing need to explore innovative approaches that can be utilized either independently or in combination with conventional treatments to yield positive outcomes. Traditional herbal medicine holds great potential in providing novel anti-urolithiatic therapies due to the bioactive nature of its extracts and constituents. The literature indicates the historical utilization of several herbal plants with potential efficacy against kidney stones (Evan *et al.*, 2010). Plant extracts have exhibited promising outcomes in managing urolithiasis, attributed to their various biological activities such as diuretic, antioxidant, crystallization inhibition, lithotriptic, analgesic, and anti-inflammatory properties. Numerous chemical components derived from

medicinal plants, including triterpenoids, berberine, gallotannin, curcumin, quercetin, catechin, and rutin, have demonstrated beneficial effects against urolithiasis. Among these, betulin, linoleic acid, lupeol, α -amyirin, β -amyirin, and other pentacyclic triterpenoids have been extensively investigated. In animal models, structurally related pentacyclic triterpenes lupeol and betulin have shown anti-urolithiatic activity. Lupeol is postulated to reduce calcium levels by enhancing or restoring renal tubular reabsorption. The anti-urolithiatic and diuretic properties of triterpenoids from various plants have been observed in response to these potential mechanisms (Joy *et al.*, 2012). Another pentacyclic triterpenoid, oleanolic acid, exhibits potent diuretic effects and shares structural similarities with lupeol and betulinic acid. Additionally, the antioxidant constituents of these plants aid in ameliorating kidney damage induced by crystals or oxalates (Vidya and Varalakshmi, 2000).

The presence of diverse pentacyclic triterpenoids, including betulinic acid, ursolic acid, oleanolic acid, α -amyirin, and β -amyirin, has been documented in the stem bark of *Madhuca longifolia*, as well as in the leaves of *Catharanthus roseus* and *Salvia officinalis*. However, the anti-urolithiatic potential of these plants remains unexplored. Therefore, the objective of this study was to assess the impact of hydroalcoholic extracts from these plants on *in-vitro* urolithiasis using nucleation and aggregation assays.

Materials and Methods

Chemicals:

For the study, analytical-grade chemicals and other solutions were used.

Collection of plants:

The bark of *Madhuca longifolia* was collected from in and around the Semariya region, Dist. Rewa, Madhya Pradesh, India, during February 2022, and leaves of *Catharanthus roseus* were harvested from the local area of Indore, India, during February 2022, while leaves of *Salvia officinalis* were collected from the local vendor of Indore,

India, in January 2022.

Extraction:

The air-dried bark of *Madhuca longifolia* (sample 1), as well as the air-dried leaves of *Catharanthus roseus* (sample 2) and *Salvia officinalis* (sample 3), underwent defatting with petroleum ether. The resulting powdered materials were individually subjected to maceration using a hydroalcoholic solvent (30:70 v/v). The concentrated extracts were filtered twice using Whatman filter paper No. 1 and subsequently placed in a vacuum desiccator to ensure complete removal of the solvent. Following this, the residues were stored in separate airtight glass vials at 4°C until further use.

Preliminary phytochemical screening:

The hydroalcoholic extract of the above plants was tested using conventional phytochemical methods for the presence of various phytochemicals like glycosides, terpenoids, saponins, sterols, flavonoids, phenols, tannins, alkaloids, carbohydrates, and proteins.

In vitro urolithiatic activity test by nucleation method:

The extracts' *in vitro* anti-urolithiatic activity was examined in terms of their ability to prevent the production of CaOx crystals both with and without inhibitors (standard drug and extracts).

Preparation of sample/standard:

A freshly prepared 1 mg/ml distilled water solution of cystone was used as the standard. 1 mg of the test samples (samples 1, 2, and 3) were taken with ethanol to make a 1 mg/ml stock solution. Different concentrations (100-1000 µg/ml) of samples 1, 2, and 3 were taken from a stock solution in a set of test tubes; later, ethanol was added to make the volume 1 ml. Through the use of the nucleation test, the impact of extracts on the production of CaOx crystals was assessed. In 0.5 M Tris and 0.15 M NaCl buffer, a solution of 5 mM CaCl₂ and 7.5 mM sodium oxalate was made (pH 6.5). The extracts and cystone were diluted in the ethanol at various concentrations (100-1000

µg/ml). The extracts and cystone were diluted in equal amounts (100, 250, 500, 750, and 1000 µg/ml) and combined with 3 ml of CaCl₂ and 3 ml of sodium oxalate solution. 1 ml of sodium oxalate solution was added to each set to start the crystallisation process. The test mixtures were then incubated for 30 min at 37°C in a boiling water bath and cooled down to room temperature. Finally, the absorbance of the mixtures was measured at 620 nm using a UV-visible spectrophotometer (Systronic 2202), using the control with no test solution, and the percentage inhibition was calculated.

For the control, 3 ml of CaCl₂ was mixed with 3 ml of sodium oxalate solution and incubated for 30 min in the water bath at 37°C. The absorbance of the control was measured against a Tris-NaCl buffer (as a blank) at 620 nm. The percentage inhibition of anti-urolithiasis activity of extracts and standards was calculated using the formula:

$$\% \text{ Inhibition} = [(Ab \text{ of control} - Ab \text{ of sample} / Ab \text{ of control} \times 100]$$

In vitro anti-urolithiatic activity test by aggregation assay:

With a few minor adjustments, the technique was identical to that explained by Hess *et al.* (1994). The purpose of this second experiment was to determine how plant extracts affected the renal system's ability to dissolve already-formed stones.

Preparation of sample/ standard:

A freshly prepared 1 mg/ml distilled water solution of cystone was used as the standard. 1 mg of the test samples (1, 2, and 3) and the standard were taken with ethanol to make a 1 mg/ml stock solution. Different concentrations of samples 1, 2, and 3 (100-1000 µg/ml) were taken from a stock solution in a set of test tubes, and ethanol was added to make the volume 1 ml. A spectrophotometric experiment was used to measure the CaOx crystals' rate of aggregation. The sodium oxalate and CaCl₂ solutions were mixed to form CaOx monohydrate (COM) crystals at a concentration of 50 mM/l. Both solutions were then heated to 60°C. The solutions were allowed to evaporate overnight after being kept at

Table 1: Nucleation assay of Samples 1, 2 and 3

S. No.	Concentration (µg/ml)	Mean ± SD				% Inhibition			
		Std.	Sample 1	Sample 2	Sample 3	Std.	Sample 1	Sample 2	Sample 3
1.	100	0.432±0.001	0.568±0.001	0.654±0.001	0.715±0.002	41.49	23.02	11.47	3.12
2.	250	0.308±0.002	0.428±0.001	0.556±0.001	0.654±0.001	58.33	41.98	24.65	11.38
3.	500	0.284±0.004	0.313±0.001	0.402±0.002	0.543±0.004	61.49	57.60	45.51	26.41
4.	750	0.174±0.002	0.232±0.001	0.347±0.001	0.447±0.001	76.39	68.57	53.00	39.41
5.	1000	0.089±0.003	0.167±0.001	0.222±0.001	0.346±0.001	87.90	77.42	69.98	53.09
IC ₅₀		32.72	54.49	65.08	59.81				

37°C. A final concentration of 1 mg/ml of the COM crystals was achieved by dissolving them in 0.5 ml of 0.05 mM Tris buffer and 0.5 ml of 0.15 mM NaCl solution at pH 6.5. A 620 nm absorbance measurement was made. The rate of aggregation was calculated by comparing the slope of turbidity in the presence of extract vs. control. Readings at 620 nm were taken when the extract was incubated at a temperature of 37°C at different concentrations (ranging from 100 to 1000 µg/ml) with 3 ml of COM crystal solution. The % inhibition of crystal aggregation was calculated using the equation below:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

By graphing the percentage inhibition concerning control against the treatment concentration, the samples (1, 2, and 3) concentration for 50% inhibition (IC₅₀) was determined.

Results

Nucleation assay:

In the present investigation, the *in vitro* anti-urolithiasis activity of extracts 1, 2, and 3 was evaluated by nucleation assay (Table 1). Cystone was used as the standard.

The estimation of crystal production in the nucleation assay was based on the turbidity of the

solution. The absorbance of the extracts was subtracted from the control absorbance to determine the extent of crystal formation. Increasing the concentration of the extracts in the nucleation experiment resulted in a significant reduction in absorbance. The percentage inhibition of nucleation for samples 1, 2, and 3 extracts was 77.42, 69.98, and 53.09, respectively, while the standard exhibited a percentage inhibition of 87.90 at a dose of 1000 µg/ml (Table 1, Fig. 1). The coefficient of regression (r²) for the dose-dependent increase in percentage inhibition of nucleation by the extracts was 0.97, 0.98, and 0.99, respectively. The IC₅₀ values for the plant extracts (samples 1, 2, 3) were 54.49, 65.08, and 59.81, respectively, whereas for cystone it was 37.72.

Aggregation assay:

In the aggregation assay, the *in vitro* anti-urolithiasis activity of samples 1, 2, and 3 was evaluated (Table 2). The assay involved testing the concentration-dependent inhibition of the extracts at concentrations of 100, 250, 500, 750, and 1000 µg/ml. Similarly, cystone was used as a standard, exhibiting concentration-dependent inhibition at the same concentrations.

Aggregation plays a significant role in the formation of stones, emphasizing its importance in stone genesis. The inhibitory effect on crystal

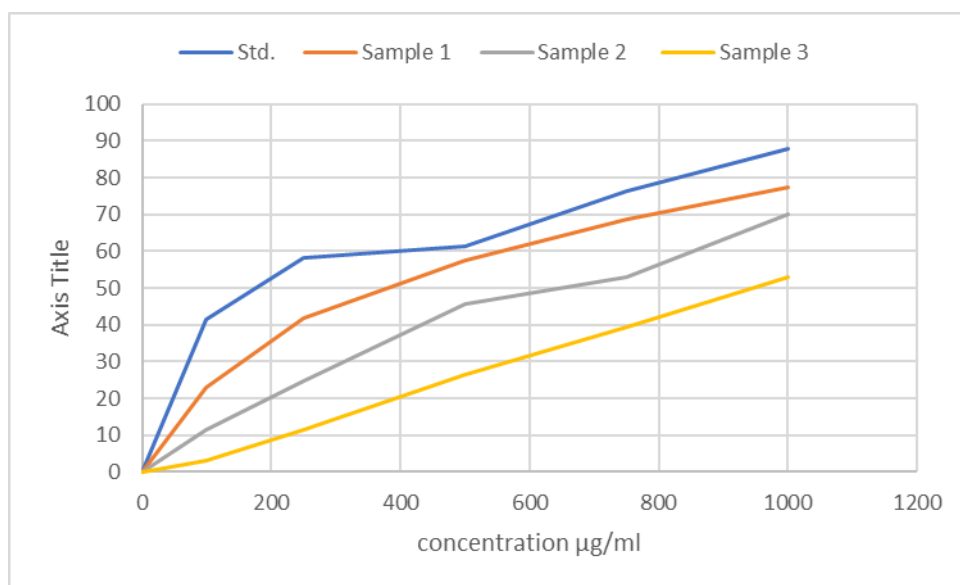


Fig. 1: Effects of control, *Madhuka longifolia* bark extract (sample 1), *Catharanthus roseus* leaf extract (sample 2), *Salvia officinalis* leaf extract (sample 3) and cystone (standard) at 100, 250, 500, 750, and 1000 µg/ml on % inhibition of nucleation.

Table 2: Aggregation assay of Samples 1, 2, and 3

S. No.	Concentration (µg/ml)	Mean ± SD				% Inhibition			
		Std.	Sample 1	Sample 2	Sample 3	Std.	Sample 1	Sample 2	Sample 3
1.	100	0.478±0.003	0.745±0.002	0.653±0.002	0.725±0.003	37.42	2.45	14.45	5.02
2.	250	0.363±0.002	0.664±0.001	0.544±0.004	0.643±0.002	52.40	13.01	28.73	15.76
3.	500	0.225±0.004	0.576±0.004	0.426±0.004	0.526±0.002	70.48	24.54	44.24	31.14
4.	750	0.123±0.005	0.443±0.002	0.327±0.003	0.475±0.003	83.84	42.01	57.21	37.73
5.	1000	0.058±0.002	0.323±0.003	0.146±0.004	0.356±0.005	92.45	57.64	80.87	53.32
IC ₅₀		35.5	51.82	57.90	72.58				

aggregation exhibited a dose-dependent relationship, where an increase in extract concentration led to a higher percentage inhibition. To assess the degree of crystal aggregation, the decrease in absorbance at 620 nm was utilized as an indirect parameter. In the aggregation assay, the percentage inhibition of sample 1 extract was 57.64%, sample 2 extract was 80.87%, and sample 3 extract was 53.32%. In comparison, the percentage inhibition of cystone at a dose of 1000 µg/ml was 92.45% (Table 2, Fig. 2). The coefficient of regression (r^2) for the dose-dependent increase in percentage inhibition of aggregation by the extracts was 0.98. The IC₅₀

values for plant extracts (samples 1, 2, and 3) were 51.82, 57.90, and 72.58, respectively, while for cystone it was 35.52.

Discussion

A comprehensive approach to managing urolithiasis involves both prevention and treatment of crystallization events. Unlike conventional medicine, which often targets a single aspect of urolithiatic pathophysiology, herbal treatments have demonstrated efficacy at multiple stages of urolithiasis. Herbal remedies possess various mechanisms of action, including diuretic effects, inhibition of crystallization,

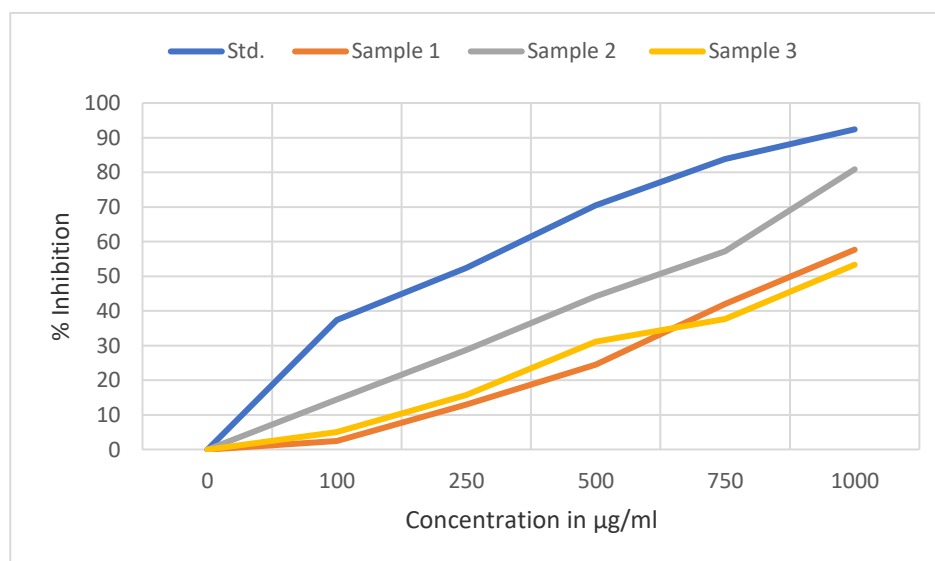


Fig. 2: Effects of control, *Madhuka longifolia* bark extract (sample 1), *Catharanthus roseus* leaf extract (sample 2), *Salvia officinalis* leaf extract (sample 3) and cystone (standard) at 100, 250, 500, 750, and 1000 µg/ml on % inhibition of aggregation.

lithotriptic activity, as well as anti-inflammatory, antioxidant, and antimicrobial properties (Yadav *et al.*, 2011). *Madhuca longifolia*, commonly known as the Buttercup tree or Mahua, holds significant economic value in India. Its bark, leaves, flowers, fruits, and seeds have been extensively utilized in traditional Indian medicine. The tree features rough, dark bark and reaches a height of approximately 10-15 meters. Literature highlights numerous biological activities associated with *Madhuca longifolia*, including antibacterial, antioxidant, anti-inflammatory, anti-diarrheal, skin disease management, anti-ulcer, analgesic, nephroprotective, anti-hyperglycaemic properties, as well as wound healing activity (Chaiyarit and Thongboonkerd, 2012). Historically, the bark of *Madhuca longifolia* has been employed in the treatment of conditions such as tonsillitis, rheumatism, ulcers, and bleeding (Devi *et al.*, 2021). Scientific literature indicates that the bark contains a rich concentration of pentacyclic triterpenoids, including amyirin, lupeol, oleanolic acid, betulinic acid, and ursolic acid. Therefore, it is evident that the bark of *Madhuca longifolia* is a notable source of pentacyclic triterpenoids.

However, no available data currently exist regarding its potential as an anti-urolithiatic agent (Chandra, 2001; Agrawal *et al.*, 2012).

Catharanthus roseus, belonging to the family Apocynaceae, is an annual sub-herbaceous plant that can reach a height of 1 m. It is commonly known as Periwinkle or Vinca rosea. The leaves of this plant are arranged in opposite pairs, measuring 2.5-9.5 cm in length and 1-3.5 cm in width, with an oval to oblong shape. They are hairless and exhibit a glossy green appearance. *Catharanthus roseus* is known to synthesize a diverse array of secondary metabolites, including pentacyclic terpenoids, alkaloids, flavonoids, saponins, steroids, and cyanogenic glycosides. Throughout history, *Catharanthus roseus* has been employed for the treatment of various ailments. Traditional medicinal uses include the management of menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, cancer, menstrual disorders, skin diseases, and it is also recognized for its antiviral, anticancer, and antioxidant properties (Nayak and Pereira, 2006). While alkaloidal secondary metabolites are abundant in *Catharanthus roseus*,

recent studies have uncovered the presence of pentacyclic triterpenoids, such as ursolic acid, betulinic acids, oleanolic acid, α -amyrin, and β -amyrin, within the plant's leaves. However, the potential of *Catharanthus roseus* against urolithiasis remains uncertain and requires further investigation (Kabesh *et al.*, 2015).

Salvia officinalis, commonly known as sage, garden sage, or common sage, is an herb of great commercial value belonging to the Lamiaceae family. It is highly esteemed due to its essential oils and diverse therapeutic properties. Published findings have demonstrated various pharmacological effects of sage, including anti-inflammatory, anti-proliferative, anti-obesity, anti-diarrheal, and anti-bacterial activities (El Euch *et al.*, 2019). Traditional medicine has long utilized sage species for multiple purposes such as pain management, prevention of oxidative stress, inhibition of free radical damage, angiogenesis, inflammation, as well as treatment of bacterial and viral infections. Phytochemical studies conducted on *S. officinalis* extracts have revealed the presence of diterpene, triterpenoid, and flavonoid compounds, with ursolic acid and oleanolic acid identified as the primary constituents.

Previous research on nucleation inhibition has postulated that reducing the bioavailability of minerals required for crystal growth can impede the aggregation of crystals. In this regard, studies have demonstrated that concealing crystal binding sites in renal epithelial cells and other crystal types can effectively prevent aggregation, consequently inhibiting the growth of unhealthy crystals (Adrar *et al.*, 2016). Notably, hydroalcoholic extracts derived from *Madhuka longifolia*, *Catharanthus roseus*, and *Salvia officinalis* significantly inhibited the formation and aggregation of calcium oxalate (CaOx) crystals at varying concentrations. However, when compared to the outcomes of cystone, the most notable result was observed with the bark extract of *Madhuka longifolia* and the leaf extract of *Catharanthus roseus*.

The present study investigates the presence of pentacyclic triterpenoids in substantial quantities in the selected parts of three plants. Extensive research has highlighted the significant anti-urolithiatic activity associated with pentacyclic triterpenoids. Nonetheless, there is currently no scientific evidence available to establish the anti-urolithiatic activity of these specific plants.

In the future, it is recommended to expand the scope of *in vitro* investigations to include *in vivo* studies. Alternatively, formulation studies utilizing the aforementioned plant extracts could be conducted with the aim of discovering a novel herbal remedy for the treatment of these debilitating conditions. However, to determine the active ingredients, optimal dosage, quality control measures, potential interactions, and side effects of these plants, further investigations are warranted.

Numerous herbs have been found to possess properties that inhibit crystallization, while their antioxidant characteristics contribute to a reduced risk of renal cell urolithiasis. Despite the widespread use and promising potential of herbal medicine, additional research is necessary to comprehensively understand the mechanisms of action and to provide effective and safe alternatives for anti-urolithiatic treatment (Ahmed *et al.*, 2016; Jalpa *et al.*, 2016).

Conclusion:

The primary findings of the present study revealed that among the three plant extracts tested, the extract derived from *Madhuka longifolia* demonstrated the highest efficacy in inhibiting crystallization based on the nucleation assay. Conversely, *Catharanthus roseus* extract exhibited the most effective inhibition of aggregation in pre-formed crystals. However, it should be noted that *in vitro* tests merely serve as an initial testing approach and do not offer a mechanistic understanding of the *in vivo* observations. The remarkable nucleation and aggregation inhibitory effects of the extracts from *Madhuka longifolia* and *Catharanthus roseus* may be attributed to their

rich content of pentacyclic triterpenoid compounds. Consequently, our research provides substantial support for the continued utilization of *Madhuka longifolia* and *Catharanthus roseus* as remedies for kidney stones. Nevertheless, in order to establish these plants as herbal treatments for kidney stones, further investigations encompassing *in vivo* research and the isolation and identification of the specific phytochemicals responsible for their actions must be undertaken.

References

- Adrar N, Oukil N and Bedjou F. (2016) Antioxidant and antibacterial activities of *Thymus numidicus* and *Salvia officinalis* essential oils alone or in combination. *Ind Crops Prod.* 88(10):112-119.
- Agrawal S, Kulkarni GT and Sharma VN. (2012) Antimicrobial and anti-inflammatory activities of bark of four plant species from Indian origin. *Webmed Central Pharm Sci.* 3(10): 1-4.
- Ahmed S, Hasan MM and Mahmood ZA. (2016) *In vitro* urolithiasis models: An evaluation of prophylactic management against kidney stones. *J Pharmacogn Phytochem* 5(3): 28-35.
- Alelign T and Petros B. (2018) Kidney stone disease: an update on current concepts. *Adv Urol.* 2018(2): 1-12.
- Chaiyarit S and Thongboonkerd V. (2012) Changes in mitochondrial proteome of renal tubular cells induced by calcium oxalate monohydrate crystal adhesion and internalization are related to mitochondrial dysfunction. *J Proteome Res.* 11(6): 3269-3280.
- Chandra D. (2001) Analgesic effect of aqueous and alcoholic extracts of *Madhuca longifolia* (Koeing). *Indian J Pharmacol.* 33(2): 108-111.
- Devi AT, Yashaswini N, Zameer F and Prasad MN. (2021) Experimental urolithiasis model to assess phyto-fractions as anti-lithiatic contributors: a herbaceutical approach. *BioRxiv.* <https://doi.org/10.1101/2021.06.01.446538>.
- El Euch SK, Hassine DB, Cazaux S, Bouzouita N and Bouajila J. (2019) *Salvia officinalis* essential oil: chemical analysis and evaluation of anti-enzymatic and antioxidant bioactivities. *S Afr J Bot.* 120(1): 253-260.
- Evan AP. (2010) Physiopathology and etiology of stone formation in the kidney and the urinary tract. *Pediatr Nephrol.* 25(5): 831-841.
- Hess B, Zipperle L, Takkinen R, Ackermann D and Jaeger P. (1994) Inhibition of calcium oxalate crystal aggregation by Tamm-Horsfall glycoprotein depends on Citrate. In: *Urolithiasis 2*, (eds.) Ryall R., Bais R., Marshall V.R., Rofo A.M., Smith L.H. and Walker V.R., Springer, Boston, MA.
- Jalpa R, Moteriya P and Chanda S. (2016) Effect of decoction extracts of some medicinal plants on calcium oxalate crystallization. *Asian J Pharm Clin Res.* 9(6): 70-76.
- Joy JM, Prathyusha S, Mohanalakshmi S, Kumar AP and Kumar CK. (2012) Potent herbal wealth with litholytic activity: a review. *Int J Innova Drug Dis.* 2(2): 66-75.
- Kabesh K, Senthilkumar P, Ragunathan R and Kumar RR. (2015) Phytochemical analysis of *Catharanthus roseus* plant extract and its antimicrobial activity. *Int J Pure App Biosci.* 3(2): 162-172.
- Madan B and Ahmad S. (2018) *In vitro* inhibition of calcium oxalate nucleation by extract-based fractions of aerial parts and roots of *Aerva lanata* (Linn.) Juss. ex Schult. *Indian J Pharm Sci.* 79(6): 957-964.
- Nayak BS and Pereira LMP. (2006) *Catharanthus roseus* flower extract has wound-healing activity in sprague dawley rats. *BMC Complement Altern Med.* 6(1):1-6.
- Santhoshi B, Chidrawar VR and Rao VU. (2015) A Brief review on the potential medicinal plants and screening models of urolithiasis. *Int J Appl Biol Pharm Technol.* 6(1): 37-45.
- Selvam R, Kalaiselvi P, Govindaraj A, Murugan VB and Kumar AS. (2001) Effect of *A. lanata* leaf extract and *Vediuppu chunnam* on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. *Pharmacol Res.* 43(1): 89-93.
- Vidya L and Varalakshmi P. (2000) Control of urinary risk factors of stones by betulin and lupeol in experimental hyperoxaluria. *Fitoterapia* 71(5): 535-543.
- Yadav RD, Jain SK, Alok S, Mahor A, Bharti JP and Jaiswal M. (2011) Herbal plants used in the treatment of urolithiasis: a review. *Int J Pharm Sci Rev Res.* 2(6): 1412-1420.