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ABSTRACT - Introduction and Aim: The etiology of urolithiasis is complex with various factors such as supersaturation, inflammation, oxidative stress and microbial infection due to E. coli, Pseudomonas spp. and urease-splitting bacteria etc., contributing to stone formation. The present article analyses the antiurolithiatic properties of four medicinal plants viz. Anneslea fragrans, Mallotus philipensis, Magnolia hodgsonii and Bauhinia variegata in comparison with Cystone. Materials and Methods: The methanolic extracts were studied for nucleation assay, aggregation assay, 2, 2-diphenylpicrylhydrazyl (DPPH) assay, ferric reducing antioxidant potential (FRAP) assay, proteinase inhibition assay, and antimicrobial studies against two-gram negative bacteria viz. Escherichia coli and Pseudomonas aeruginosa, and one-gram positive bacterium, Staphylococcus aureus. Results: The present investigation indicated that Anneslea fragrans exhibit significant anti-nucleation, anti-aggregation properties, though marginally weaker than Cystone, but have higher anti-inflammatory and anti-microbial properties as compared to Cystone. All the plant extracts exhibit significant antioxidant properties with IC_{50} values of 1.95, 1.9, 2.14 and 1.85, and ascorbic acid equivalent (AAE in mg/g) values of 0.395, 0.75, 0.349 and 0.72, significantly higher anti-inflammatory property with IC_{50} values of 420.75, 646.16, 408 and 940.93 for proteinase inhibition assay of Anneslea fragrans, Mallotus philipensis, Magnolia hodgsonii and Bauhinia variegata respectively, and significant antimicrobial properties when compared with Cystone, a polyherbal formulation. Conclusion: The current study demonstrated the therapeutic potentials of the plant extracts in ameliorating factors that cause kidney stones, and can be further investigated to identify their bioactive components.

KEYWORDS: Urolithiasis, medicinal plants, *Anneslea fragrans, Mallotus philipensis, Magnolia hodgsonii and Bauhinia variegata.*

Introduction:

Kidney stone, also known as urolithiasis, is a prevalent medical condition that affects large section of global population. Around 12% of the world's population are afflicted with kidney stones at some point in their lives (1). More than 60% of urinary stones are said to contain calcium oxalate (CaOx), more than 40% contain calcium phosphate, and only 4% have magnesium-ammonium-phosphate (struvite) stones, which are an aggregate of crystals, bacteria, and protein matrix (2, 3). The prevalence of urolithiasis has dramatically increased recently in both industrialized and developing nations. Urine supersaturation with calcium and

oxalate that resulted in crystallization, have been suggested to be one of the main causes of urinary stones (4). Research findings have suggested a correlation between microbial infection of kidney and urolithiasis (5).

The etiology and process involved in renal stone development are multifaceted. It consists of supersaturation of urine, crystal nucleation, growth, aggregation, and renal retention (6). Recent studies have suggested that urolithiasis should be considered a systemic disorder as multiple factors interacted and contributed to this underlying medical condition (7).The molecular process that causes calcium oxalate stone development is currently not fully understood. Various studies have reported that damages to renal tubular cells could take place due to exposure to high oxalate and CaOx crystals (8, 9). Injury of renal cells could have resulted in higher affinity for adhesion of crystals (10). Macromolecules that induce inflammation and fibrosis were observed to show enhanced expression and/or synthesis due to deposition of CaOx crystals in the kidney (11-13). Crystal deposition in the renal tissues has been demonstrated to be closely linked to the generation of free radicals (14). Rats supplemented with kidney stone-promoting diets showed rise in lipid peroxidation and decline in antioxidant capability (15). Therefore, it is evident that medicinal plants or its active principles that can prevent these processes can play significant role in bringing about effective medical remedy towards urolithiasis. A number of physiological properties of medicinal plant extracts, including diuretic, lithotriptic, analgesic, and anti-inflammatory properties, may contribute to their capacity to prevent urolithiasis (16). As the etiology of urolithiasis is complex, the present study explore different parameters such as inhibition potency on crystal nucleation and aggregation, antimicrobial, anti-inflammatory and antioxidant properties of four medicinal plants viz. *Anneslea fragrans, Mallotus philipensis, Magnolia hodgsonii* and *Bauhinia variegata* in comparison with Cystone.

Materials and methods

Collection of plant materials

The leaves of *Anneslea fragrans, Bauhinia variegata* and *Magnolia hodgsonii*, and barks of *Mallotus philipensis* were collected from Chandel District, Manipur and were duly identified by Taxonomist in Manipur University. The materials were properly washed in running water and dried under shade. The dried plant materials were ground and properly stored for extraction of methanolic extracts.

Preparation of solvent extracts

Methanolic extracts were obtained using Soxhlet apparatus. The total extracting time was adjusted to 24 h, using 250 ml of methanol, at 40°C. The viscous semisolid crude extracts were obtained using a rotary vacuum evaporator. The crude extracts obtained were stored at 4 °C for later use.

Nucleation assay

Nucleation assay was performed based on the methodology given by Hennequin *et al.*, 1992 with few modifications (17).

Methodology

A solution of sodium oxalate and calcium chloride was made with final concentrations of 0.5 and 3 mmol/l, respectively, in a pH 5.5 buffer that contains 0.15 mol/l of NaCl and 0.05 mol/l of Tris. 200 µl of different concentrations of plant extracts were mixed with 1.9 ml of calcium chloride solution and incubation was done at 37 ºC for 30 min in a water bath. Crystallization reaction was initiated by addition of 1.9 ml of sodium oxalate solution. The optical density of the solution was observed at 620 nm, and percentage inhibition was calculated as follow;

$$\% \text{ Inhibition} = \{(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}\} \times 100$$

Aggregation assay

The aggregation assay was carried out using a slightly modified methodology of Hess et al., 1989 (18).

To prepare seeds of CaOx crystals, 50 mmol/l solutions of $CaCl_2$ and $Na_2C_2O_4$ were combined together, heated to 60 ºC in a water bath for one hour, and then incubated overnight at 37 ºC. Following drying, 0.8 mg/ml of CaOx crystal solution was prepared in a buffer containing 0.05 mol/l Tris-HCl and 0.15 mol/l NaCl at pH 6.5. 3 ml of CaOx solution was mixed with 1 ml of aliquots (100–1000 µg/ml) of the sample, vortexed, and incubated for 30 min at 37 ºC. The optical density of the final mixture was measured at 620 nm, and the percentage inhibition of aggregation was estimated using the following formula;

% Inhibition = {(Absorbance of control - Absorbance of sample) / Absorbance of control} × 100

Antioxidant study

2, 2-diphenylpicrylhydrazyl (DPPH) assay

Blois's method was followed in determining the plant extract's antioxidant activity against DPPH (19). Ascorbic acid was used as the reference standard. Using the following formula, the percentage of DPPH radical scavenging activity of plant extracts was determined;

% DPPH radical scavenging activity = {($A_0 - A_1$)/A_0} × 100

Where, where A_0 represents the absorbance of control and A_1 represents the absorbance of sample.

Standard preparation

The quantification of antioxidant activity of plant extracts were determined by 2, 2-diphenylpicrylhydrazyl (DPPH) assay using Ascorbic acid (2 mg/ml) as standard solution. Ascorbic acid in different concentrations (200, 400, 600, 800 & 1000 µl) were prepared using methanol. To 1 ml of all different concentration prepared, 3 ml of DPPH solution (6mg DPPH in 100ml methanol) was added. The mixture was properly mixed, and incubation was done for 30 min in the dark at room temperature. The optical density was measured at 517 nm with spectrophotometer. The antioxidant potential is inversely proportional to the absorbance of the reaction mixture. A calibration curve showing the percentage of DPPH scavenged against the standard concentration was developed.

Sample preparation

A 2 mg/ ml of plant extracts were prepared, and different concentrations (200, 500, 1000 µl) of extract were prepared by using methanol. To 1 ml of extracts, 3 ml of DPPH was added, thoroughly mixed, and incubated in the dark at room temperature for 30, and the optical density was recorded at 517nm with spectrophotometer. The inhibition percentage was plotted against concentration, and IC_{50} was calculated using the graph.

Ferric Reducing Antioxidant Potential (FRAP) assay

Standard preparation

The Ferric Reducing Power (FRAP) assay was used to measure the antioxidant activity, with ascorbic acid (1 mg/ml) as a standard. Standard was prepared in different concentration (200,

400, 600, 800, 1000 µl) of ascorbic acid. 1 ml of sodium phosphate buffer (0.2 mol; pH=6.6) and 1ml of 1% potassium ferricyanide were added to 0.1 ml of each different concentrations of ascorbic acid prepared, and the mixture was then incubated at 50°C for 20 min. Subsequently, the mixture was mixed with 1 ml of 10% trichloroacetic acid (TCA) and centrifuged for 10 min at 3000 rpm. Then, 1.5 ml of distilled water and 0.1 ml of 0.1% ferric chloride ($FeCl_3$) were combined with 1.5 ml of supernatant. Then, 10 min incubation was done at room temperature, and the optical density was measured at 700 nm with spectrophotometer.

Sample preparation

A 2 mg/ ml of extracts was soaked overnight in methanol for the assay. 0.5 ml of extracts was made up to 1 ml with solvent methanol and mixed thoroughly. Then 1 ml of 1% potassium ferricyanide and 1 ml sodium phosphate buffer (0.2 mol; pH=6.6) were added to the mixture, and kept for incubation for 20 min at 50°C. To this, 1 ml of 10% TCA was added, and centrifuged for 10 min at 3000 rpm. To 1.5 ml supernatant, 0.1% ferric chloride ($FeCl_3$) and 1.5 ml of distilled water was added. Then, incubation was done for 10 min at room temperature, and its optical density was measured at 700 nm with spectrophotometer. High absorbance signifies higher reducing potential. The reducing potentials were expressed in mg equivalents of Ascorbic Acid Equivalent per g of sample (mg AAE/g dry weight of samples).

Antimicrobial study

The antimicrobial activity was analysed by using disc diffusion method against two gram positive bacteria viz. *Pseudomonas aeruginosa and Escherichia coli*, and *Staphylococcus aureus*, a gram positive bacterium. Nutrient agar (medium) and Plate count agar (medium) were autoclave-sterilized after being dissolved in distilled water at 121°C at 15psi for 15 min, and then cooled at room temperature. After being poured into petri-plates, the agar medium was left to cool at room temperature until it solidified. The Nutrient agar and Plate count agar plates were inoculated with standardized inoculum of *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Using sterile disc of filter paper the surface of solidified media was inoculated by soaking each disc onto sample extract having concentration 2 mg/ml. Plates were kept in room temperature for diffusion of sample for 30 min. Standard plate was made with ampicillin, penicillin and vancomycin. Then, incubation was done for 24 h at 37 °C, and the zone of inhibition was assessed.

Anti-inflammatory activity

Proteinase Inhibition assay

The proteinase inhibition assay was done using the method developed by Sakat et al with few modifications (20). The reaction mixture consisted of 3 ml of 0.07 mg of trypsin along with 1 ml of 20 mmol Tris HCl buffer of pH 7.4, as well as 1 ml of a test sample with varying concentrations ranging from 50 to 600 µg/ml. The mixture underwent incubation at 37 ^{o}C for 5 min. Following this, 1 mL of 1% (weight/volume) casein solution was introduced. The incubation period was extended by 20 min. A volume of 2 ml perchloric acid with a concentration of 70% was introduced into the reaction mixture in order to halt its progress. After centrifugation, the absorbance was recorded at 210 nm, using buffer as a blank. The experiment was conducted in triplicates to ensure accuracy and reliability of the results. The inhibition percentage of proteinase activity was measured as follows;

% inhibition of denaturation = 100 × (1 – Absorbance of sample/ Absorbance of control)

Results

Anti-nucleation and anti-aggregation studies

The results showed that among the four plants being studied *Anneslea fragrans* showed significant anti-nucleation and anti-aggregation potential with value of 41.86% and 56.67% when compared with Cystone, a polyherbal formulation, as depicted in table 1 and 2, and figure 1 and 2 respectively.

Samples	**% inhibition of CaOx nucleation**
Anneslea fragrans	41.86%
Mallotus philipensis	1.78%
Magnolia hodgsonii	22.78%
Bauhinia variegata	7.62%
Cystone	51.19%

Table 1: Percentage CaOx nucleation inhibition activity.

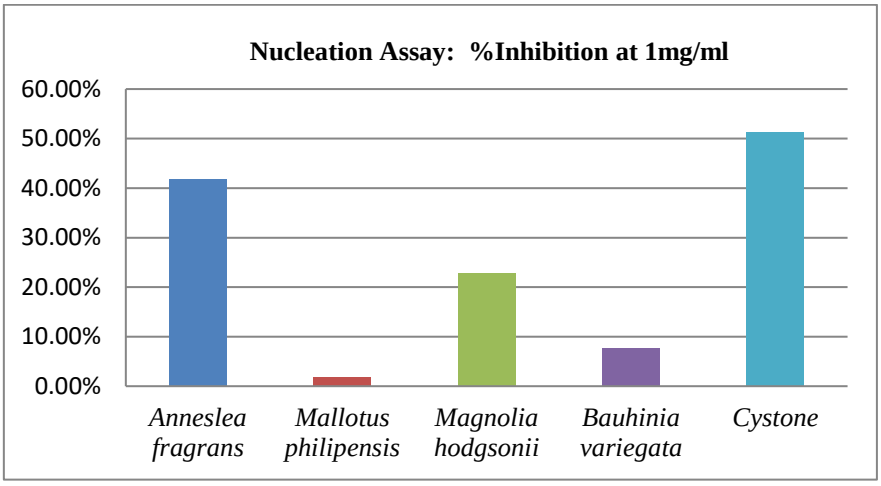

Figure 1: Graphical representation of CaOx nucleation inhibition activity of different plant extracts.

Samples	% inhibition of CaOx nucleation
Anneslea fragrans	56.67%
Mallotus philipensis	0.60%
Magnolia hodgsonii	36%
Bauhinia variegata	15.90%
Cystone	57.51%

Table 2: Percentage CaOx aggregation inhibition activity of different methanolic extracts.

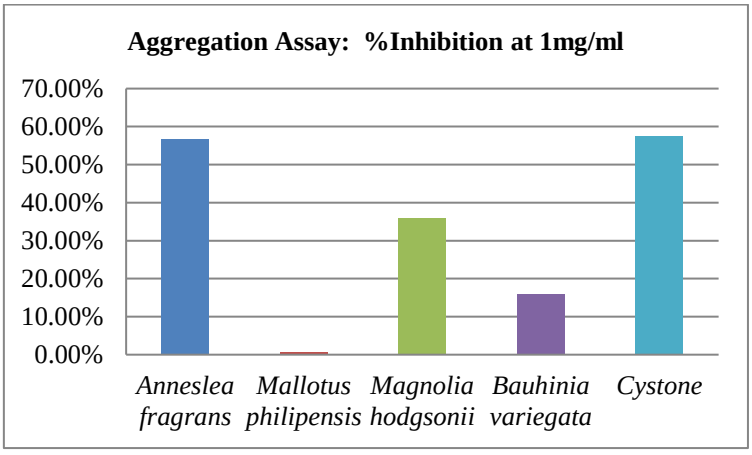

Figure 2: Graphical representation of CaOx aggregation inhibition activity of different extracts.

Antioxidant studies

2, 2-diphenylpicrylhydrazyl (DPPH) Assay

The DPPH study using ascorbic acid (2 mg/ml) as standard showed that all the plant extracts showed significant antioxidant properties. Among the plant extracts studied *Bauhinia*

variegata showed greater antioxidant property with IC_{50} value of 1.85 as depicted in table 3 and figure 3. The remaining other medicinal plants also showed comparable yet lesser antioxidant properties as compared to Cystone.

Samples	**IC_{50} values**
Anneslea fragrans	1.95
Mallotus philipensis	1.9
Magnolia hodgsonii	2.14
Bauhinia variegata	1.85
Cystone	1.4

Table 3: IC_{50} values of different plant extracts in comparison with that of Cystone.

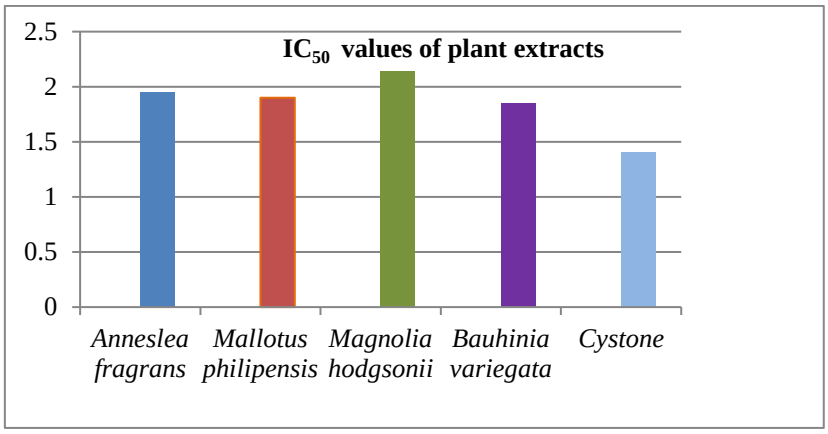

Figure 3: Graphical representation IC_{50} values of different plant extracts in comparison with Cystone.

Ferric Reducing Antioxidant Potential (FRAP) Assay

The FRAP assay showed that *Mallotus philipensis* and *Bauhinia variegata* showed significant antioxidant properties in comparison with Cystone with AAE values of 0.895, 0.95 and 0.92 respectively as depicted in table 4 and Figure 4.

Samples	**Volume of sample (µl)**	**Absorbance (700nm)**	**Concentration of test samples (µg)**	**Equivalent concentration of ascorbic acid mg AAE/g of dry weight of sample (AAE mg/g)**
Anneslea fragrans	500	0.0345	395	0.395
Mallotus philipensis	500	0.070	750	0.75
Magnolia hodgsonii	500	0.0299	349	0.349
Bauhinia variegata	500	0.067	720	0.72
Cystone	500	0.0845	895	0.895

Table 4: Data showing Absorbance values at 700 nm and Ascorbic acid equivalent (AAE) in mg/g for FRAP analysis.

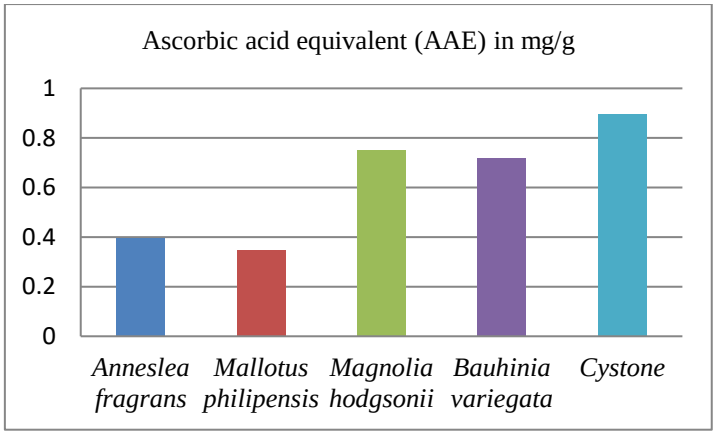

Figure 4: Graphical representation of Ascorbic acid equivalent (AAE) in mg/g for different plant extracts.

Anti-inflammatory study

The anti-inflammatory study indicated that *Magnolia hodgsonii* and *Anneslea fragrans* have higher anti-inflammatory potential as compared to Cystone, as presented by their IC_{50} values of 408 and 420.75 respectively, as shown in table 5 and figure 5. Similarly, all the studied medicinal plants showed greater proteinase inhibition potential as compared to Cystone.

% Inhibition of Proteinase: IC_{50} values	
	IC_{50} values
Anneslea fragrans	420.75
Mallotus philipensis	646.16
Magnolia hodgsonii	408
Bauhinia variegata	940.93
Cystone	1078.95
Aspirin@ 100ug/ml	81.57

Table 5: IC_{50} values of different samples

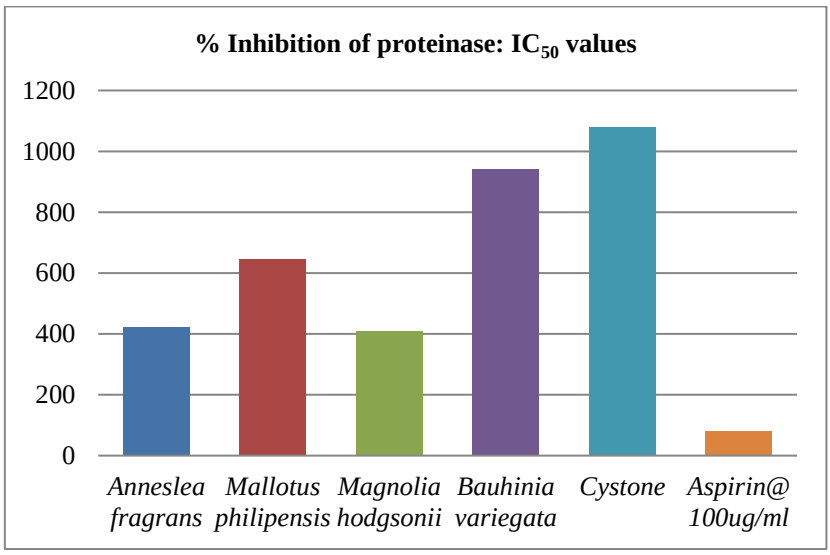

Figure 5: Proteinase inhibition percentage represented in IC_{50} values

Anti-microbial studies

Anti-microbial studies indicated that all the selected plants showed activities against both gram negative and gram positive bacteria. Among the selected plants *Magnolia hodgsonii* appeared to have better anti-microbial activity against *Staphylococcus aureus*, a gram positive bacterium. The zone of inhibition against gram negative bacterium *Pseudomonous aeruginosa* was much of the same range, though it was slightly higher in Cystone as depicted in table 6 and figure 6.

Zone of Inhibition (in mm)			
Samples	*E.coli* (-ve)	*Pseudomonous aeruginosa* (-ve)	*Staphylococcus aureus* (+ve)
Anneslea fragrans	8	14	16
Mallotus philipensis	15	12	16
Magnolia hodgsonii	13	12	18
Bauhinia variegata	8	13	12
Cystone	6	15	15

Table 6: Zone of inhibition (mm) of different samples.

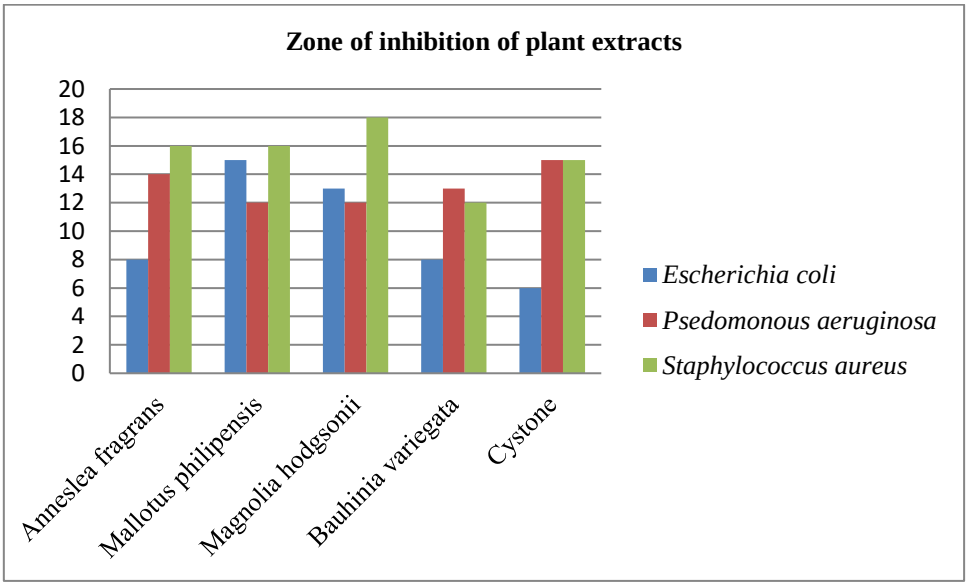

Figure 6: Graphical representation of antimicrobial activities of different samples analyzed.

Discussion

The present study highlighted the medicinal properties of four medicinal plants used in Urolithiasis. The results indicated the important scientific authentication of medicinal plants traditionally used in stone disease. The current study emphasizes on the importance of multifactorial approach in evaluation of medicinal plant extracts for urolithiasis, as the mechanism of stone formation is complex. Urine supersaturation being one of the main prerequisites for crystals formation in the urinary tract, the present study on anti-nucleation and anti-aggregation studies demonstrated that *Anneslea fragrans* has remarkable inhibition

properties, having inhibition values of 41.86% and 56.67% respectively when compared with Cystone. These findings suggested that the presence of bioactive components that prevent CaOx crystal nucleation and aggregation. The fact that Cystone contains a variety of therapeutic medicinal plants may account for its marginally greater inhibitory values, each of which contributes to its medical efficacy.

Reactive oxygen species (ROS) are extremely reactive molecules with unpaired electrons that have the ability to damage and alter the chemical composition of nucleotides, proteins, lipids, and carbohydrates. (21,22). Various experimental and clinical data indicated that the renal epithelial cells exposed to elevated concentrations of CaOx and calcium phosphate (CaP) crystals produce ROS, that led to injury and inflammation. Studies have also shown that rats with hyperoxaluria and CaOx nephrolithiasis produce lipid peroxides in their urine and renal tissue (23, 24). It has been demonstrated that oxalate and crystal deposition-induced oxidative stress can be prevented by antioxidants such vitamin E, selenium and catechin (25, 26). The present studies on 2, 2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity showed that *Bauhinia variegata, Mallotus philipensis and Anneslea fragrans* showed significant antioxidant properties with IC_{50} values of 1.85, 1.9 and 1.95 respectively. The Ferric reducing antioxidant power (FRAP) assay also indicated significant antioxidant properties with AAE values of 0.75 mg/g and 0.72 mg/g for *Mallotus philipensis* and *Bauhinia variegata* respectively, as compared to Cystone with AAE value of 0.895 mg/g, which are indicative of their protective and preventive attributes towards kidney stone formation.

Crystal formation has been suggested to cause activation of inflammation pathways (27). Research has linked kidney cell damage, inflammation, cell loss and fibrosis to crystal deposition (28). Studies have indicated the crucial role of reactive oxygen species (ROS) in stone formation as both agents of inflammation and injury, and signalling molecules (29-31). The anti-inflammatory study demonstrated that *Magnolia hodgsonii* and *Anneslea fragrans* showed higher anti-inflammatory potential as compared to Cystone, as showed by their IC_{50} values of 408 and 420.75 respectively. Similarly, all the studied medicinal plants have better proteinase inhibition potential as compared to Cystone.

E. coli and *Pseudomonas spp* were implicated to be the most commonly isolated bacteria from stone cultures, responsible for struvite stone formation (32-34). Therefore, the antimicrobial study was conducted to analyze their activities against these microbes. The results on antimicrobial studies using two gram positive bacteria viz. *Escherichia coli* and *Pseudomonas*

aeruginosa and one gram positive bacterium viz. *Staphylococcus aureus* suggested that all the samples were effective against the tested microbes. The tested samples turned out to be even more effective against *Staphylococcus aureus,* a gram positive bacterium. Among the plant extracts, *Magnolia hodgsonii* was found to be most effective against *Staphylococcus aureus,* when compared to Cystone, whereas *Mallotus philipensis* was found to have similar activitity with Cystone.

Conclusion

The etiology of stone formation is complex involving many factors, such as urine supersaturation, inflammation, oxidative stress and microbial infection. Consequently, it becomes crucial to address each contributing element. Since plant extracts contain different types of phytochemicals, they are one of the best candidates to look for effective bioactive compounds for urolithiasis. Moreover, various medicinal plants, each highly effective against one or more contributing factors, can be properly analysed and used for production of polyherbal formulation like Cystone. In our present study, among the four plants the anti-nucleation and anti-aggregation properties were only slightly lesser than that of Cystone, which could be due to its additive increase owing to presence of many plants in Cystone. Similar conclusion can be made for antioxidant studies and antimicrobial study. All the plant extracts exhibit greater anti-inflammatory potential than Cystone, however *Anneslea fragrans* and *Magnolia grandiflora* showed significantly greater efficacy among them. Thus, it can be concluded that the search for potent bioactive chemicals against urolithiasis will be able to proceed with greater efficiency by screening more traditionally used medicinal plants.

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Conflict of Interest

Nil

Funding

Nil

References

1. Sofia NH, Manickavasakam K, Walter TM. Prevalence and risk factors of kidney stone. GJRA 2016; 5:183–187.

2. Pak CY, Poindexter JR, Adams-Huet B et al. Predictive value of kidney stone composition in the detection of metabolic abnormalities. Am J Med 2003; 115:26-32.

3. Flannigan R, Choy WH, Chew B, et al. Renal struvite stones pathogenesis, microbiology, and management strategies. Nat Rev Urol 2014; 11:333-41.

4. Coe FL, Parks JH, Asplin JR. The pathogenesis and treatment of kidney stones. N Engl J Med 1992; 327:1141-52.

5. Flannigan R, Choy WH, Chew B, et al. Renal struvite stones--pathogenesis, microbiology, and management strategies. Nat Rev Urol 2014;11:333-41.

6. Tavasoli S, Taheri M. Vitamin D and calcium kidney stones: A review and a proposal. Int. Urol. Nephrol. 2019; 51: 101–111.

7. Cicerello E, Ciaccia M, Cova GD, Mangano MS. The new patterns of nephrolithiasis: What has been changing in the last millennium? Arch Ital Urol Androl 2021, 93, 195–199.

8. Khan SR. Calcium oxalate crystal interaction with renal epithelium, mechanism of crystal adhesion and its impact on stone development. Urol Res. 1996; 23: 71–9.

9. Tsujihata M, Miyake O, Yoshimura K, Tsujikawa K, Tei N, Okuyama A. Renal tubular cell injury and fibronectin. Urol Res. 2003; 31: 368–73.

10. Wiessner JH, Hasegawa AT, Hung LY, Mandel GS, Mandel NS. Mechanisms of calcium oxalate attachment to injured renal collecting duct cells. Kidney Int. 2001; 59: 637–44.

11. Hackett RL, Shevock PN, Khan SR. Alterations in MDCK and LLC-PK: cells exposed to oxalate and calcium oxalate monohydrate crystals. Scanning Microsc. 1995; 9: 587–96.

12. Hackett RL, Shevock PN, Khan SR. Madin-Darby canine kidney cells are injured by exposure to oxalate and to calcium oxalate crystals. Urol Res. 1994; 22: 197–204.

13. Koul H, Kennington L, Honeyman T, Jonassen J, Menon M, Scheid C. Activation of c-myc gene mediates the mitogenic effects of oxalate in LLC-PK1 cells, a line of renal epithelial cells. Kidney Int. 1996; 50: 1525–30.

14. Trump BF, Berezesky IK, Laiho KU, Osornio AR, Mergner WJ, Smith MW. The role of calcium in cell injury. Scanning Electron Microsc. 1980; 2: 437-62.

15. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV. Effects of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol. 2006;105:306–311.

16. Joy JM, Prathyusha S, Mohanalakshmi S, Kumar PAVS and Kumar A CK . Potent herbal wealth with litholytic activity: a review. Inter J Innovat Drug Discovery. 2012; 2(2): 66-75.

17. Hennequin C, Lalanne V, Daudon M, Lacour B and Dru Èeke T. A new approach to studying inhibitors of calcium oxalate crystal growth. Urological Research 1993; 21: 101-8.

18. Hess B, Nakagawa Y and Coe FL: Inhibition of calcium oxalate monohydrate crystal aggregation by urine proteins. American Journal of Physiology. 1989; 257: F99-F106.

19. Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature.* 1958; 181:1199–1200.

20. Sakat S, Juvekar AR, Gambhire MN. In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. Int J Pharm Pharm Sci. 2010; 2:146–155

21. Dröge W. Free radicals in the physiological control of cell function. Physiol Rev 2002; 82:47-95.

22. Kamata H, Hirata H. Redox regulation of cellular signalling. Cell Signal 1999;11:1-14.

23. Toblli JE, Ferder L, Stella I, et al. Effects of angiotensin II subtype 1 receptor blockade by losartan on tubulointerstitial lesions caused by hyperoxaluria. J Urol 2002; 168:1550-5.

24. Thamilselvan S, Hackett RL, Khan SR. Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. J Urol 1997; 157:1059-63.

25. Santhosh Kumar M, Selvam R. Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. J Nutr Biochem. 2003; 14(6):306-313.

25. Thamilselvan S, Khan SR, Menon M. Oxalate and calcium oxalate mediated free radical toxicity in renal epithelial cells: effect of antioxidants. Urol Res. 2003; 31(1):3-9.

26. Khan SR, Glenton PA, Byer KJ. Modeling of hyperoxaluric calcium oxalate nephrolithiasis: experimental induction of hyperoxaluria by hydroxy-L-proline. Kidney Int. 2006; 70:914-23.

27. Mulay SR, Kulkarni OP, Rupanagudi KV, Migliorini A, Darisipudi MN, et al. Calcium oxalate crystals induce renal inflammation by NLRP3-mediated IL-1beta secretion. J Clin Invest. 2013; 123: 236-246.

28. Khan SR. Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissueculture studies. Clin Exp Nephrol. 2004; 8:75-88.

29. Khan SR. Role of renal epithelial cells in the initiation of calcium oxalate stones. Nephron Exp Nephrol. 2004; 98:e55-60.

30. Khan SR. Hyperoxaluria-induced oxidative stress and antioxidants for renal protection. Urol Res. 2005; 33:349-57.

31. Khan SR. Renal tubular damage/dysfunction: key to the formation of kidney stones. Urol Res. 2006; 34:86-91.

32. Tavichakorntrakool R, Prasongwattana V, Sungkeeree S, et al. Extensive characterizations of bacteria isolated from catheterized urine and stone matrices in patients with nephrolithiasis. Nephrol Dial Transplant, 2012; 27:4125-30.

33. Thompson RB, Stamey TA. Bacteriology of infected stones. Urol. 1973;2: 627-33.

34. Golechha S, Solanki A. Bacteriology and chemical composition of renal calculi accompanying urinary tract infection. Indian J Urol. 2001; 17:111-7.