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Evaluation of Anti-urolithiatic Properties of Some Medicinal Plants from Manipur

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ABSTRACT: The prevalence of urinary stones or urolithiasis is about 12% in India and has a higher prevalence of 15% in northern India. Approximately 98% of the patients are found to develop urolithiasis within 25 years of the first attack. Unfortunately, despite advances in treatment, there is still no satisfactory treatment that can cure urinary stones or reduce their recurrence. Medicinal plants have been known for thousands of years and are valued worldwide for being rich in chemical compounds that cure and prevent diseases. Medicinal plants are becoming increasingly popular because they are effective, non-toxic and have no side effects. Many studies have been reported in Manipur on the use of herbal medicines in the treatment of urolithiasis or kidney stones. The present article aims to analyze the anti-urolithiatic properties of four medicinal plants *viz., Anneslea fragrans, Mallotus philipensis, Magnolia hodgsonii* and *Bauhinia acuminate* which were documented for their use in traditional methods of treatment of kidney stone in Manipur. The selected plants were studied for their calcium oxalate (CaOx) anti-nucleation and anti-aggregation assay, 2, 2-diphenylpicrylhydrazyl (DPPH) assay, proteinase inhibition assay and anti-microbial study by agar disc diffusion method against *Escherichia coli*, a gram negative bacterium, and *Staphylococcus aureus,* and a gram positive bacterium. The studies were done in comparison with Cystone, which is a commercially available herbal formulation used in urinary stone disease. The results showed that amongst the four selected plants *A. fragrans* exhibited the highest percentage of Ca Oxnucleation and aggregation inhibition values of 41.86% and 56.67% respectively, which is approximately equivalent to the efficacy of Cystone. DPPH assay indicated that *B. acuminata* exhibited highest antioxidant property with IC_{50} value of 1.85mg/mL, which is substantially equal to that of Cystone. Moreover, *B. acuminata* and *A. fragrans* showed proteinase inhibition potential with IC_{50} values of 408 µg/mL and 420.75 µg/mL respectively, significantly superior to Cystone, but less effective than Aspirin. Antimicrobial studies indicated that *M. hodgsonii* was found to have higher zone of inhibition against *S. aureus,* whereas *M. philipensis* showed higher zone of inhibition against *E. coli*. The results of the present study showed important properties of the selected medicinal plants against urolithasis. It also calls for urgent need for scientific evaluation of important medicinal plants documented for urolithiasis.

Keywords: *Anneslea fragrans, Bauhinia acuminata*, kidney stone, *Magnolia hodgsonii, Mallotus philipensis.*

INTRODUCTION

Urolithiasis, also known as nephrolithiasis or kidney stone, is one of the most common diseases affecting a large number of people worldwide. 12% of the population worldwide suffers from kidney stones at some point in their lives (Sofia *et al.*, 2016). Despite its longstanding prevalence in humans, urolithiasis poses significant medical and public health challenges (Singh and Sailo 2013). Urolithiasis is often a recurrent and lifelong disease with a recurrence rate of 50% within 5-10 years and 75% within 20 years (Eisner and Goldfarb 2014). It is estimated that 1-15% individuals suffer from kidney stone formation at some point during their lifetime, and the prevalence and incidence of kidney stone is reported to be increasing worldwide (Romero *et al*., 2010; Morgan and Pearle 2016). The high prevalence and the recurrent nature of urolithiasis have resulted in an increased economic burden on healthcare systems (Sutherland *et al.*, 1985). The incidence of kidney stones has increased in both developed and developing countries in the last few years. Their prevalence in India also reflects worldwide prevalence and stands at approximately 12% (Guha *et al*., 2019; Kakkar and Kakkar 2021) and is relatively more common in the northern part of India, where it is 15%. Among the five types of kidney stones *viz.*, calcium oxalate (CaOx), carbapatite ($Ca_{10}(PO_4)_6CO_3$), urate ($C_5H_4N_4O_3$), struvite ($MgNH_4PO_4{\cdot}6H_2O$) and brushite ($CaHPO_4{\cdot}2H_2O$) stones, the calcium oxalate variety of renal stones is the most common, constituting 60% of all these stones (Shin *et al.*, 2021). CaOx stones are formed when calcium combines with oxalate in the urine, resulting in crystalline structures (Sellaturay and Fry 2008). Overweight and hypertension have been associated with CaOx stones (Assimos, 2006). Overweight patients, in particular, are at an elevated risk of developing uric acid stones. Furthermore,

patients with diabetes and hypertension had a higher frequency of uric acid stone (Schwaderer and Wolfe 2017; Ferraro *et al.*, 2020). Pakistan (18%) and India (23%) had a more significant rate of struvite stones (Halinski *et al.*, 2023). Therefore, it is essential to consider various risk factors in conjunction with biochemical stone analysis when managing patients with infectious stones for optimal treatment. The cause and mechanisms of kidney stone formation is multifactorial. It includes urine supersaturation, followed by crystal nucleation, growth, aggregation, and retention in the kidney (Tavasoli and Taheri 2019). The first stage of kidney stone formation is super saturation of urine, which occurs when the concentration of certain substances in urine (such as calcium, oxalates and phosphates) exceeds the solubility limit. This causes the urine to become supersaturated, creating a favourable environment for crystal formation. Under these conditions, the excess fluid no longer dissolves and begins to clump together to form small crystal particles (Ratkalkar and Kleinman 2011). Over time, crystals can aggregate into larger pieces, which can lead to stone formation. The aggregation of crystals can be influenced by many factors, including the concentration and composition of stone-forming salts, the pH of the urine, and the presence of organic or inorganic molecules that can act as promoting agents or inhibitors of crystal aggregation (Evan *et al*., 2007; Khan, 2006). Crystal formation has been suggested to cause activation of inflammation pathways (Mulay *et al*., 2013). Crystal deposition is associated with renal cell injury, cell loss, inflammation and fibrosis (Khan, 2004a). Research using animal models and tissue cultures showed that elevated levels of oxalate, calcium oxalate, and calcium phosphate crystals triggered inflammatory responses in renal cells through reactive oxygen species (Khan, 2013). Antioxidants such as vitamin E, catechin and selenium have been shown to provide protection against oxidative damage caused by oxalate and crystal formation (Santhosh and Selvam 2003; Khan *et al*., 2006). Studies have also link kidney stones with microbial infection of the urinary system (Flannigan *et al*., 2014). Evidences have indicated the role of microbes such as *E. coli, S. aureus* and *Pseudomonas spp* in the formation of kidney stones (Tavichakorntrakool *et al*., 2012; Thompson and Stamey 1973; Golechha and Solanki 2001), with developed countries typically having lower prevalence of only 4% of urinary stones, while in developing countries, it's about 10–20% of urinary stones (Karki and Leslie 2021). The formation of these stones appears to be heavily influenced by the composition of urine and the interaction between bacteria and various components of the urinary system (Karki and Leslie 2021). Bacteria in infected urine produce an enzyme called urease, which breaks down urea into ammonia and carbon dioxide that results in alkaline environment, forming struvite stones or infection stones. Another way in which bacterial infection can contribute to stone formation involves an increase in crystal adherence (Trinchieri, 2014).

Despite numerous treatments and synthetic drugs, urinary stones are still difficult to treat. Furthermore, the significant recurrence rate of 31%-75% after initial cessation makes the treatment of urinary stones difficult (Anderson, 2002). The number of medical visits and drug costs required for the treatment of this disease constitute a heavy burden for patients and society. Urolithiasis is treated with various medical treatments depending on the size and location of the stone. With the help of some drugs, stones smaller than 5 mm are passed through the urine. However, large stones can be treated with extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL). Unfortunately, none of these control methods are effective in treating stones as they are associated with unwanted side effects (Butterweck and Khan 2009). Kidney stones have also been shown to be associated with the development of hypertension, diabetes and heart disease (Khan, 2012).

Medicinal plants have been recognized for thousands of years and are of great value worldwide as rich medicinal agents for disease and immunity (Sharma *et al*., 2008). Medicinal plants are becoming increasingly popular due to their beneficial effects, non-toxicity and absence of side effects (Pifferi *et al*., 1999). Different studies have reported the traditional use of medicinal plants for treatment of urolithiasis or urinary stone disease in Manipur (Shyamkiran, 2022; Ahmed and Singh 2011; Mikawlrawng *et al*., 2014). Isolating bioactive compounds from these plants that can inhibit CaOx crystal formation presents a promising strategy for preventing urolithiasis. Different studies have documented the use of *A. fragrans, M. philipensis, M. hodgsonii* and *B. acuminate* in traditional method for treatment of urinary stone disease (Imotomba and Lisham 2011; Lokendrajit *et al*., 2011; Lokesh *et al*., 2011). The leaves of *A. fragrans* was reported for its use for treatment of kidney stone by people from Khangshim, Chandel district, Manipur (Lokesh *et al*., 2011). The decoction of the barks of *M. philippensis* is used in urinary tract stone problem (Lokendrajit *et al*., 2011). The decoction of barks or leaves of *B. acuminate* is used to cure stone in bladder (Lokendrajit *et al*., 2011). The leaves of *M. hodgsonii*is used in stone disease (Imotomba and Lisham 2011). However, the detail scientific or laboratory based studies of these plants for their anti-urolithiatic properties are lacking. Therefore, the present study explores different parameters such as inhibition potency on CaO xnucleation and aggregation, anti-microbial, anti-oxidant and anti-inflammatory activities of the four selected medicinal plants.

MATERIALS AND METHODS

Collection of plant materials. The leaves of *A. fragrans, B. acuminata* and *M. hodgsonii*, and barks of *M. philipensis* were collected from Chandel District, Manipur. The materials were properly washed in running water and dried under shade. The dried materials were grinded and kept in an airtight container for the extraction of methanolic extracts.

Preparation of methanolic extracts. Methanolic extracts were obtained using Soxhlet apparatus. 50 g of dried powder sample was placed inside a muslin cloth thimble into the main chamber of a Soxhlet apparatus. The entire extraction duration was 24 h, utilizing 250 mL of solvent, with the solvent continuously refluxing over the sample at 40°C. The viscous semisolid crude extracts obtained using rotary vacuum evaporator were kept at 4°C in dark containers for further use. The percentage yield of extract was calculated from the equation given below.

% Yield of extract = $(W_1 \times 100) / W_2$

Where, W_1 is the weight of the extract residue after solvent removal and W_2 is the weight of initial dried sample powder used.

Nucleation assay. Nucleation assay was performed as per the method described by Hennequin *et al*. (1992), with some minor modifications. Solutions of Calcium chloride ($CaCl_2$) and Sodium oxalate ($Na_2C_2O_4$) were prepared at a final concentration of 3 mM/L and 0.5 mM/L respectively, in a buffer containing 0.05 M/LTris and 0.15 M/LNaCl at pH 5.5.1.9 mL of $CaCl_2$ solution was mixed with 200 µL of different concentrations (100–1000 µg/mL) of the plant extracts prepared in 99.98 % methanol, and incubated for 30 min at 37 ºC in a water bath. Crystallization was started by adding 1.9 mL of sodium oxalate solution. The optical density (OD) of the solution was measured at 620 nm, and the percentage inhibition was calculated by using the given formula:

% Inhibition = {(Absorbance of Control – Absorbance of Sample) / Absorbance of Control} × 100

Aggregation assay. Aggregation assay was performed as per the method described by Hess *et al*. (1989) with some minor modifications. 250 mL of $CaCl_2$ and $Na_2C_2O_4$ solutions (50 mM/L each) were mixed together, heated to 60ºC in a water bath for 1h, and then incubated overnight at 37ºC to prepare seeds of CaOx crystals. After drying, CaOx crystal solution (0.8 mg/mL) was prepared in a 0.05 M/LTris-HCl and 0.15 M/LNaCl buffer (pH 6.5). 1 mL of different concentrations (100-1000 µg/mL) of the samples prepared in 99.98 % methanol, were added to 3 mL of CaOx solution, vortexed, and then incubated at 37 ºC for 30 min. OD of the final mixture was read at 620 nm wavelength, and percentage inhibition of aggregation was calculated using the formula given below.

% Inhibition = {(Absorbance of Control – Absorbance of Sample) / Absorbance of Control} × 100

Antioxidant study

2, 2-diphenylpicrylhydrazyl (DPPH) assay. The antioxidant activity of the plant extract was determined as per the method described by Blois, 1958. To 1 mL of different concentrations (100-2000 µg/mL) of methanolic extracts prepared in 99.98 % methanol, 3mL of DPPH solution (6mg DPPH in 100 mL methanol) were added. The mixture was shaken vigorously and kept in room temperature for 30 min in the dark, and the OD was recorded at 517nm using spectrophotometer. Lower absorbance of the reaction mixture indicates higher DPPH radical scavenging activity. Ascorbic acid was used as standard. Percentage of DPPH radical scavenging activity (RSA) was calculated by using the equation given below.

% DPPH radical scavenging activity = {(Absorbance of Control – Absorbance of Sample) / Absorbance of Control} × 100

The antioxidant activity was expressed in IC_{50}, defined as the concentration of the extract required to achieve 50% inhibition of DPPH, by plotting a linear regression graph between percentage RSA and concentration of the extracts.

Anti-inflammatory activity

Proteinase Inhibition assay. The proteinase inhibition assay was conducted based on method developed by Sakat *et al*. (2010). The reaction mixture of 2 mL containing 0.07 mg tryps in, 1 mL of 20 mM/LTris HCl buffer of pH 7.4, as well as 1 mL of test samples of varying concentrations (50-600 µg/mL) was prepared in 99.98 % methanol. The mixture was incubated at 37ºC for 5 min. Following this, 1 mL of a 1% (weight/volume) casein solution was added and incubated for another 20 min. Then, 2 mL of 70%perchloric acid was added to the reaction mixture, followed by centrifugation. The absorbance of the resulting supernatant was measured at 210 nm using spectrophotometer. Aspirin (100 ug/mL) was used as the standard. The percentage of proteinase inhibitory activity was calculated using the formula given below.

% inhibition of denaturation = {(Absorbance of Control – Absorbance of Sample)/ Absorbance of Control} × 100

Antimicrobial study

Disc diffusion method. The antimicrobial activity was analysed by disc diffusion method, with minor modifications (Bauer *et al*., 1966). The cultures of a gram positive bacterium *Staphylococcus aureus* MTCC3160 and a gram negative bacterium *Escherichia coli* MTCC68 were obtained from the Department of Microbiology, Ram Lal Anand College, University of Delhi. The bacterial cultures grown in nutrient agar medium were suspended in fresh normal saline. The turbidity of the resulting suspension was set to 0.5 McFar land turbidity to prepare a 1×10^8 bacterial/mL inoculum. Sterile Mueller Hinton plates were inoculated with the culture of the specific bacterial strains using sterile cotton swabs and kept inside laminar air flow chamber for 15 min for absorption to occur. Sterile Whatman No. 1 filter paper discs of 5 mm diameter and 0.2 mm thickness were infused with 100 µL of 2 mg/mL extract prepared in 99.98 % methanol. Methanol loaded discs were used as negative control, whereas Amphicilin (10 µg/mL), Penicilin (10 µg/mL) and Vancomycin (30 µg/mL) antibiotic discs were used as positive control. At the end of the incubation period (24 h), the antibacterial activity was evaluated by measuring the zone of inhibitions.

RESULTS AND DISCUSSION

RESULTS

Yield of extract. *A. fragrans* exhibited the highest extract yields, followed by *B. acuminata, M. philipensis, M. hodgsonii and Cystone* respectively (Table 1).

Table 1: Yield percentage of methanolic extracts of samples.

Sr. No.	Samples	Initial weight(g)	Extract weight(g)	Percentage yield
1.	*A. fragrans*	50	11.63	23.26%
2.	*B. acuminata*	50	9.99	19.99%
3.	*M. hodgsonii*	50	6.49	12.99%
4.	*M. philipensis*	50	8.38	16.77%

Anti-nucleation and anti-aggregation studies. The findings indicated that *A. fragrans* demonstrated the highest CaOx anti-nucleation and anti-aggregation abilities among the four plants, followed by *M. hodgsonii* (Table 2, 3, and Fig. 1, 2). The CaOx anti-nucleation and anti-aggregation capabilities of *A. fragrans* were found to be approximately equivalent to that of Cystone.

Table 2: CaOx nucleation inhibition activity of different extracts.

Samples	% inhibition of CaOx nucleation
A. fragrans	41.86 ± 0.005%
M. philipensis	1.78% ± 0.005%
M. hodgsonii	22.78% ± 0.004%
B. acuminata	7.62%± 0.002%
Cystone	51.19%± 0.005%

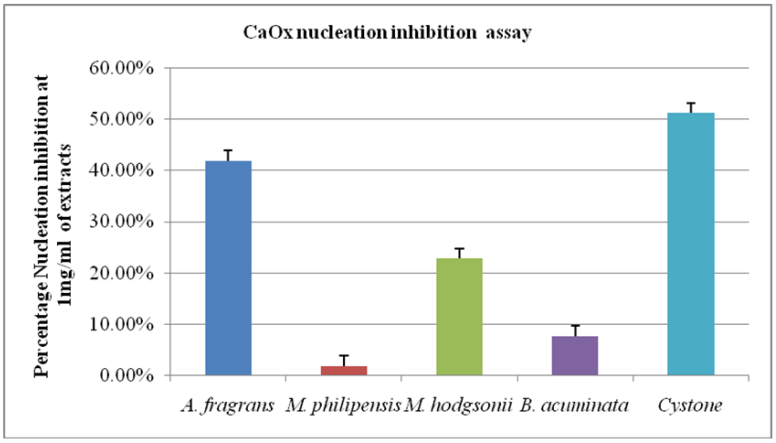

Fig. 1. CaOx nucleation inhibition activity of different extracts.

Table 3: CaOx aggregation inhibition activity of different extracts.

Samples	% inhibition of CaOx aggregation
A. fragrans	56.67±0.005%
M. philipensis	0.60 ±0.0002%
M. hodgsonii	36±0.01%
B. acuminata	15.90±0.005%
Cystone	57.51±0.01%

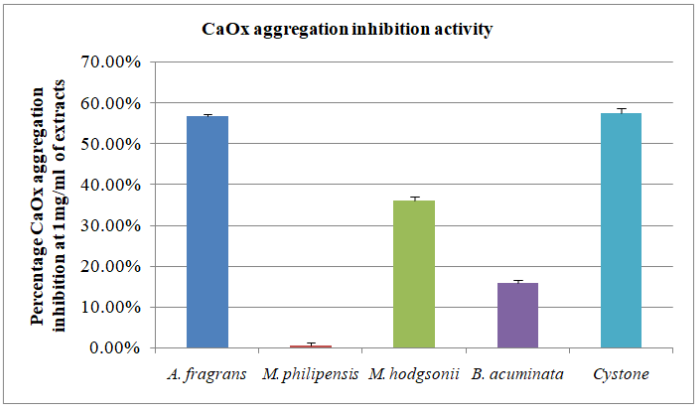

Fig. 2. CaOx aggregation inhibition activity of different extracts.

Antioxidant study

2, 2-diphenylpicrylhydrazyl (DPPH) assay. The DPPH assay with ascorbic acid (2mg/mL) as the standard revealed that all plant extracts displayed notable antioxidant characteristics. The analysis of the selected plant extracts demonstrated that *B. acuminata* exhibited better antioxidant capabilities with an IC_{50} value of 1.85 mg/mL (Table 4 and Fig. 3). The other

remaining medicinal plants exhibited similar but lower antioxidant capacities compared to Cystone. Nevertheless, ascorbic acid was the most effective out of all the samples.

Table 4: IC_{50} values of different extracts.

Samples	IC_{50} values (mg/mL)
A. fragrans	1.95±0.08
M. philipensis	1.9±0.07
M. hodgsonii	2.14±0.1
B. acuminata	1.85±0.09
Cystone	1.53±0.1
Ascorbic Acid	0.72±0.003

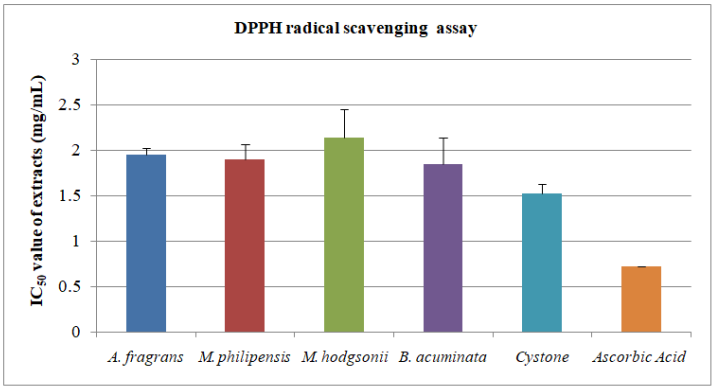

Fig. 3. IC_{50} values of different samples in comparison with Ascorbic acid.

Anti-inflammatory study. The proteinase inhibition assay showed that *A. fragrans*, *M. hodgsonii*, and *M. philippensis* have significantly better anti-inflammatory properties compared to Cystone based on their IC_{50} values of 408µg/mL, 420.75 µg/mL and 646.16 µg/mL respectively (Table 5 and Fig. 4). All the selected plants were found to exhibit higher anti-inflammatory capabilities. However, aspirin was found to be more effective than all the selected samples.

Table 5: IC_{50} of proteinase inhibition study.

% Inhibition of Proteinase: IC_{50} values (µg/mL)	
	IC $_{50}$ values
A. fragrans	420.75 ±32
M. philipensis	646.16±34
M. hodgsonii	408±25
B. acuminata	940.93±40
Cystone	1077.9±35
Aspirin@ 100µg/mL	81.57±10

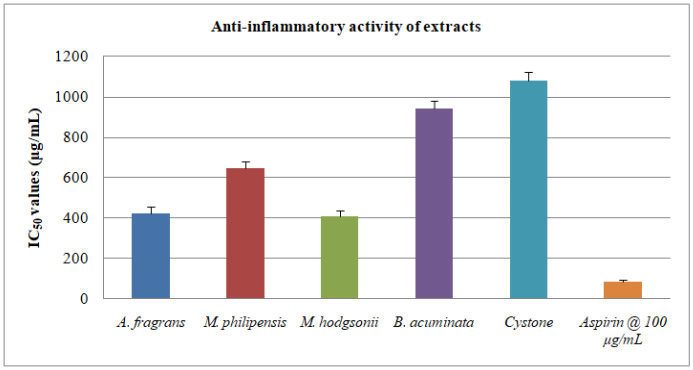

Fig. 4. IC_{50} values of anti-inflammatory study of different extracts.

Anti-microbial studies. Anti-microbial studies demonstrated that amongst the selected plants *M. philipensis* displayed marginally higher anti-microbial activity against *E. coli*, a gram negative bacterium. Nevertheless, the standard drugs proved to be much more efficient than the methanolic extracts. The zone of inhibition against *S. aureus*, a gram positive bacterium was observed to be slightly higher in that *M. hodgsonii*. The standard drug Amphicilin and Vancomycin was found to be much more effective than all the samples (Table 6, Fig. 5).

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

Zone of Inhibition (in mm)		
Samples	*E. coli*	*S. aureus*
A. fragrans	8±0.9	16±1.2
M. philipensis	15±1.8	16±1.0
M. hodgsonii	13±1.6	18±1.5
B. acuminata	8±0.7	12±0.9
Cystone	6±0.4	15±1.0
Amphicilin (10 µg/mL)	13±1.4	18±1.6
Penicilin (10 µg/mL)	8±0.5	-
Vancomycin (30 µg/mL)	18±1.5	-
Methanol (99.98%)	-	-

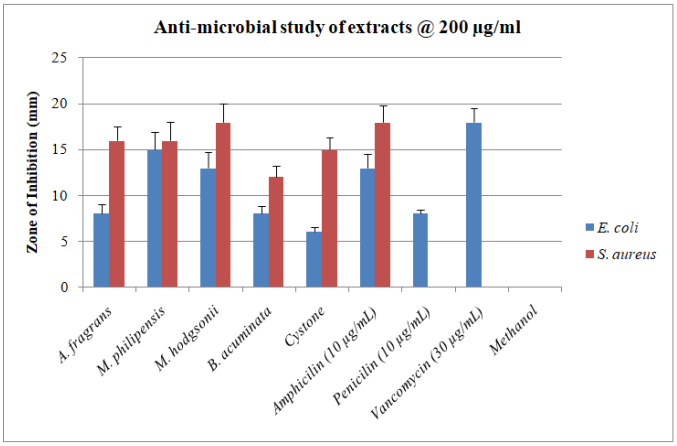

Fig. 5. Antimicrobial activities of different samples.

DISCUSSION

The cause of stone formation is complex and involves many factors such as urine oversaturation, inflammation, oxidative stress and microbial infection. Therefore, it is important to mention all programs. Plant extracts are one of the best candidates for finding bioactive compounds in the treatment of urolithiasis, as they contain a wide range of phytochemicals. This study emphasizes the importance of a simple multifactorial approach in the evaluation of herbal plants in the treatment of urinary stones. The research results provide a scientific basis for the use of *Anneslea fragrans, Mallotus philipensis, Magnolia hodgsonii* and *Bauhinia acuminate* in the treatment of kidney stones. Nucleation is the process in which free crystal forming elements combine in supersaturated urine to form crystals, which are insoluble. The process of crystallization occurs when urine becomes oversaturated, leading to the formation of solid crystals. The specific type and characteristics of the crystals depend on the substances present in the urine and the conditions prevailing during their formation (Wang *et al.*, 2021). The anti-nucleation and anti-aggregation studies indicated that *A. fragrans* exhibit notable inhibition properties, which is more or less equivalent with that of Cystone. These observations indicated the presence of bioactive compounds(s) capable of inhibiting CaOx crystal nucleation and aggregation. The slightly higher CaOx nucleation and aggregation inhibition values of Cystone may be due to the product containing various medicinal plants that contribute to its beneficial effects (Mohanty *et al.*, 2010).

Reactive oxygen species (ROS) are highly reactive molecules comprised of free radicals, atoms or molecules with unpaired electrons, and their metabolites, that can generate damage to proteins, lipids, carbohydrates and nucleotides (Dröge, 2002; Kamata and Hirata 1999). There are many studies showing that antioxidants such as vitamin E, selenium and catechins provide protection against oxidative stress caused by oxalic acid and crystal accumulation (Santosh and Selvam 2003; Khan *et al.*, 2006). The DPPH radical scavenging activity showed that *B. acuminata, M. philipensis and A. fragrans* showed significant antioxidant properties with IC_{50} values of 1.85, 1.9 and 1.95 respectively, which are marginally less than that of Cystone. The presence of antioxidant properties is indicative of their protective and preventive attributes towards kidney cell damage against ROS.

Crystal formation has been suggested to cause activation of inflammation pathways (Mulay *et al.*, 2013). Research has linked kidney cell damage, inflammation, cell loss and fibrosis to crystal deposition (Khan, 2004 b). Studies have indicated the crucial role of reactive oxygen species in stone formation as both agents of inflammation, injury, and signalling molecules (Khan, 2004b; Khan 2005; Khan 2006). The anti-inflammatory study demonstrated that *A. fragrans, M. hodgsonii*, and *M. philipensis* exhibited significantly greater anti-inflammatory potential compared to Cystone, which is crucial for preventing CaOx-induced inflammatory reactions in kidney cells.

Studies have also link kidney stones with microbial infection of the urinary system (Flannigan *et al.*, 2014). Evidences have indicated the role of microbes such as

E. coli, *S. aureus* and *Pseudomonas* spp in the formation of kidney stones (Tavichakorntrakool *et al.*, 2012; Thompson and Stamey 1973; Golechha and Solanki 2001), with developed countries typically having lower prevalence of only 4% of urinary stones, while in developing countries, it's about 10–20% of urinary stones (Karki and Leslie 2021). The antimicrobial study showed that all the selected samples demonstrated similar results as that of Cystone, although *M. hodgsonii* and *M. philipensis* displayed marginally greater abilities against *S.aureus* and *E.coli* respectively.

CONCLUSIONS

Plant extracts are among the most promising sources for urolithiasis due to the presence of various phytochemicals. As multiple factors (nucleation, aggregation, inflammation, microbial infection, and decrease in anti-oxidative potential) are involved in kidney stone development, medicinal plants that exhibit good activities against these processes can be further selected for isolation of the bioactive compounds responsible for the bioactivity. Our research indicated that the anti-urolithiatic effects of *A. fragrans* might stem from its capabilities to inhibit CaOx nucleation and aggregation, along with having anti-oxidative and anti-inflammatory characteristics. It can be suggested that the anti-urolithiatic properties of *M. hodgsonii* and *M. philipensis* could be attributed to their anti-oxidative and anti-inflammatory potentials, while that of *B. acuminata* could be due to its anti-oxidative capabilities. As CaOx nucleation and aggregation are some of the initial steps in the process of kidney stone development, it is can suggested that further analysis of *A. fragrans* that exhibit significant anti-nucleation and anti-aggregation among the selected plants, will be useful for isolation of bioactive compounds responsible for the bioactivities observed. Also, based on the observed results, where *B. acuminata* showed good antioxidant properties, *B. acuminata* and *A. fragrans* showed high proteinase inhibition potential, the present study suggested that further research on the bioactive principles of these plants will be highly beneficial in the search for anti-urolithiatic agents from plants. Similar studies can be conducted for different medicinal plants which are traditionally used in urolithiasis. However, a thorough and conclusive result necessitates multidisciplinary strategy that combines different advanced scientific methods that will significantly assist in future research on the extraction of bioactive compounds that contribute to the observed medicinal traits in these plants.

FUTURE SCOPE

Even with great clinical progress and the availability of a lengthy list of synthetic drugs, management of urolithiasis remains a major challenge. Number of follow ups required along with the cost of medication makes this disease a clinical as well as economic burden to the patients and society. Different treatment options for urolithiasis are used depending on the size and location of the calculi. With the help of some drugs, stones smaller than 5mm are forced to move through urine. But larger stones are managed through extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL). Unfortunately, neither of these management approaches is effective against the complete treatment of stones. Therefore, it is suggested that with more detail studies on medicinal plants traditional documented for the use in urolithiasis, by incorporating methods for isolation of bioactive compounds, molecular docking research, surface plasmon resonance technology (SPR), and ligand fishing methods, it will be of great benefit in the present search for novel therapeutic agents against urolithiasis.

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Conflict of Interest. None.

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