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Phytomedicine for Controlling Urolithiatic Agents

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ABSTRACT: Crystal aggregation and retention are critical events in the formation of kidney stones. Traditional plants are a valuable source of novel antibacterials and urine decrystallization agents that are associated with prevention and control of urolithiasis. Urine decrystallization and *in vitro* antibacterial activity of seventeen plant extracts were assessed. *In vitro* preparation of synthetic struvite and changes in the final weight of the stone were also determined after treatment with different ethanol extracts of the plants. Aqueous extracts of dried plant samples were used to determine their capacity to control crystallization of urine. Antibacterial susceptibilities were also determined by standard filter-disc diffusion technique. Two known antibiotics were used as positive control. Out of the seventeen plants analysed, *Cissus adnata* Roxb., *Cuminum cyminum* L., *Eupatorium birmanicum* DC., *Hibiscus sabdariffa* L., *Oxalis corniculata* L., *Piper longum* L. and *Tamarindus indica* L. significantly prevented crystal formation in urine and exhibited strong antibacterial activity against four urolithiasis inducing flora. Ethanol extracts of *H. sabdariffa* L., *T. indica* L. and *P. longum* L. showed comparatively the highest efficacies in dissolving the stone. Thus, *C. adnata*, *C. cyminum*, *E. birmanicum*, *H. sabdariffa*, *O. corniculata*, *P. longum* and *T. indica* showed promising role in prevention and control of urolithiasis.

Key words: Microbes, Inhibition zone, Urolithiasis, Decrystallization, Medicinal plants

Introduction

Urolithiasis is one of the most common diseases of the urinary tract which has been afflicting human kind since antiquity. Urolithiasis is associated with calculus formation at any level in the urinary collecting system, but calculus often arises in the kidney (Kumar et al. 1992). It occurs more frequently in men than women but rare in children (Smith, 1978), affecting approximately 12% of men and 5% of women by the age of 70. Recurrent stone formation is probably the most important problem in the after care of patients who have undergone operations for renal and ureteric calculi. Urolith formation is a multifactorial process which may relate to diet, urinary tract infection, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque and microliths, etc (Fowler, 1995).

When the urea-splitting organisms infect the urinary tract, bacteria disintegrate the urea excreted in the urine in the presence of urease enzyme, which subsequently trigger the formation of ammonia rendering the urine alkaline. In alkaline state, urine tends to contain precipitated crystals of calcium and magnesium phosphate and calcium carbonate in large amount thereby leading to a strong tendency to form calcium phosphate and calcium carbonate calculi (Chute and Suby, 1943). Bacterial infection may induce stone formation by crystal adherence. Struvite and other urinary calculi are caused by the action of bacteria on urine (Thomson and Stamey, 1973). Most of the urea-splitting organisms belong to species *Proteus* but, organisms such as *Pseudomonas, Klebsiella, Staphylococcus*, *Escherichia coli* and even *Mycoplasma* were reported to be capable of producing urease (Friedlander and Braude, 1974; Griffith et al. 1976). Robertson in 1992, has reported that infected stones were associated with the organisms like *E. coli, Proteus sp., Streptococcus, Staphylococcus*, *Pseudomonas* and *Ureaplasma urealyticum*. There are increasing evidences that have been reported that the end products of urealysis damage the glycosaminoglycan layer of the renal urothelial cells thus leading to the bacterial adherence, biofilm formation and mineral encrustation (Griffith and Osborne, 1987). Exhaustive microbiological investigations are therefore necessary to diagnose and treat the infection responsible for the stone formation.

Medicinal plants are of great economic importance in the Indian subcontinent. The documentation of traditional knowledge especially on the medicinal uses of plants in the history has provided many important drugs of the modern day (Fabricant and Farnsworth, 2001). Even today, this area holds much more hidden treasure as almost 80% of the human population in developing countries is dependent on plant resources for health-care (Farnsworth et al. 1985). Herbal medicines offer conventional treatments, providing safe and well-tolerated remedies for chronic illnesses which typically resulted from the combinations of secondary plant metabolites that are synthesized and deposited in specific parts or in all parts of the plant. Since, many of the existing synthetic drugs cause various side effects, drugs synthesized from the higher plants continue to occupy an important niche in modern medicine and play an important role in the introduction of new therapeutic agents.

A variety of plants including those used by traditional medical practitioners grow luxuriantly in Manipur (24^{o} 49´ N and 93^{o} 52´ E), a region in the north-eastern part of India, which happens to be within the Indo-Burmese mega-biodiversity hot-spot (Myers et al. 2000). Since its civilization, the living population of this region has been using various medicinal plants for the treatment of stone cases. These plants are conventionally used to prevent formation of stone as well as to dissolve and remove them from the human body.

The screening of plant extracts for antimicrobial activity is necessary to throw insight knowledge to medicinal flora and get the molecules responsible for the activity which adds value to natural resources from tropical areas (Rios and Recio, 2005). Thus, scientific investigations on the plant based indigenous medicines prepared and used by the Tribal and Meitei population of Manipur may prove to be of great pharmacological importance leading to the advent of novel drugs, which could be at par with the modern allopathic medicines in terms of efficacy, minimal side-effects and cost affordability. This led us to investigate the screening of various medicinal plants for their potential activities in prevention and control of urolithiasis.

Materials and Methods

Collection of urine samples

First voided morning mid-stream (pre-operative) urine samples were collected aseptically from 25 urolithiatic patients admitted in the Urology Department, Regional Institute of Medical Sciences, Imphal, for testing crystal formation using different plant extracts. The subjects were mostly adults (aged 20-80) chosen randomly, comprising of 14 males and 11 females. Detailed case histories, relevant information about occupation, family history of stone, onset of urolithiasis, previous urinary tract disease, age, sex, racial origin, environment, metabolic activities, dietary habit, obstructive uropathy, infection of urinary tract symptomatology (like dysuria, nocturia, hematuria, pyuria etc.), origin of backache, site of stone etc. were collected. Since the experiment does not involve human subjects directly, the approval from the ethics committee does not apply. And moreover, samples such as urine are considered as excreta and urine samples were collected after taking consent from each patient by giving proper explanation.

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Collection and extraction of plant samples

The plant samples (Table 1) were collected randomly from various places of Imphal-West (24° 37´ N and 93° 30´ E) district, and deposited in the Herbarium of Manipur University, Imphal and voucher numbers were assigned. The plant samples were oven-dried at 50 °C for (2-3) days and ground to fine powder. About 30 gm each of these powdered samples were crushed using mortar and pestle and homogenized in suitable solvent systems (distilled water/80% ethanol). The crude extracts obtained were centrifuged and then filtered through Whatmann No.1 filter paper, sterilized and stored at refrigerated conditions (4°C) for future use.

Table 1: Names of plants along with their voucher numbers and parts used

Sl. No.	Plant species (Voucher No.)	Parts used
1.	*Allium odorum* L. (002510)	Leaves
2.	*Asparagus racemosus* Willd. (Deb 253)	Roots
3.	*Averrhoa carambola* L. (Deb 2210)	Leaves
4.	*Bonnaya brachiata* Benth. (003420)	Whole plant
5.	*Cissus adnata* Roxb. (Deb 573)	Leaves
6.	*Cissus discolor* Blume. (Deb 450, 482 & 543)	Leaves
7.	*Coix lachryma jobi* L. (Mukherjee 3522)	Leaves
8.	*Cuminum cyminum* L. (Deb 2341)	Seeds
9.	*Eupatorium birmanicum* DC. (002521)	Leaves
10.	*Hedychium marginatum* C.B. Clarke (Deb 782 & 832)	Rhizomes
11.	*Hibiscus sabdariffa* L. (Deb 1377)	Outer part of the calyx
12.	*Mimosa pudica* L. (Deb 2467)	Roots
13.	*Orthosiphon spiralis* (Lour.) Merr. (Kanjilal 502)	Leaves
14.	*Oxalis corniculata* L. (Mukherjee 2808)	Whole plant
15.	*Piper longum* L. (Deb 1265)	Leaves
16.	*Pratia begoniifolia* Lindl. (00060)	Whole plant
17.	*Tamarindus indica* L. (Deb 2477)	Leaves

Determination of the effects of different plant extracts on urine crystallization

For qualitative assessment of effect on urine crystallization, 15 mL each of pre-operative urine samples were treated with 5 mL each of the plant extracts. And 20 mL of pre-operative urine sample was taken in a beaker without any treatment to serve as control. In another beaker, 15 mL of pre-operative urine was taken and treated with 5 mL of distilled water. All the samples in the experiment were taken as triplicates. The samples were then incubated for 72 hrs. at room temperature for testing crystal formation.

Anti-microbial evaluation of different medicinal plant-extracts

In the urinary system of man, infections are mostly caused by gram-negative bacteria and rarely by gram-positive bacteria. Bacterial strains namely *Proteus mirabilis* (MTCC 729), *Escherichia coli* (MTCC 729), *Pseudomonas stutzeri* (MTCC 2489) and *Klebsiella pneumoniae* sub sp. *pneumoniae* (MTCC 432) which had been reported to be responsible for urinary tract infections were selected for the present study. The antibacterial activities of the plant extracts were determined by following standard filter disc-diffusion technique. In this, the strains of bacteria were cultured on blood-agar plates. Overnight grown bacteria (1 O.D.) were thoroughly spread on blood-agar plates. The filter-discs (about 6 mm in diameter) were placed on the inoculated plates into which 50 µL each of the plant extract was added. The plates were then maintained at room temperature for about 1 hr. to allow diffusion of the plant extracts into the discs and the medium and subsequently incubated at 37°C. Inhibition was followed by measuring the diameter of the inhibition zone at the end of 24 hrs, 48 hrs, and 72 hrs. The experiments were done in replicates of three. Sensitivity of each organism to different plant extracts was assessed by observing zones of inhibition.

Preparation of synthetic struvite and its treatment using different plant extracts

When magnesia mixture [$MgCl_2$, NH_4Cl] and a little aqueous ammonia were added to a solution of sodium phosphate [Na_2HPO_4], a white crystalline precipitate of magnesium ammonium phosphate [Mg (NH_4) PO4.6H2O] was produced (Gurtu and Kapoor, 1983). This precipitate was repeatedly washed with distilled water to free the ammonium and chloride ions and its absence was confirmed through flame-test and silver-nitrate test respectively. The precipitate obtained was soluble in acetic acid and mineral acids and insoluble in dilute ammonia solution. The chemical reaction is depicted below:

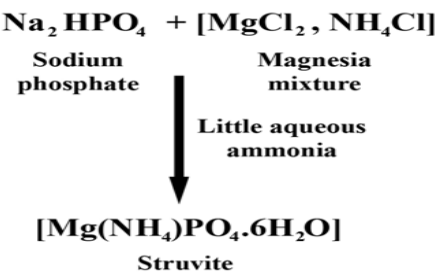


Figure (a): Synthetic struvite

This synthetically prepared struvite was treated with different quantities of ethanol extract of plants and incubated at 25 ^{o}C for 24 hours to see the effects of the plant extracts on the stone.

Results

Out of the 25 patients taken under study, 9 patients showed urine decrystallization in the control state (i.e. without any treatment of plant extract in their urine samples). So, their data were not taken into account for statistical analysis. Fig 1, shows the effects of different plant extracts on urine decrystallization and revealed that the aqueous extracts of *C. adnata*, *H. sabdariffa*, *O. corniculata* and *T. indica* have significant positive effect on urine decrystallization in all the patients. Extracts of *P. longum* was also observed to have significant positive effect on urine decrystallization in almost all the patients, but extracts of *A. carambola* showed the least efficacy on urine-decrystallization.

Fig 1: Prevention of urine crystallization using plant extracts

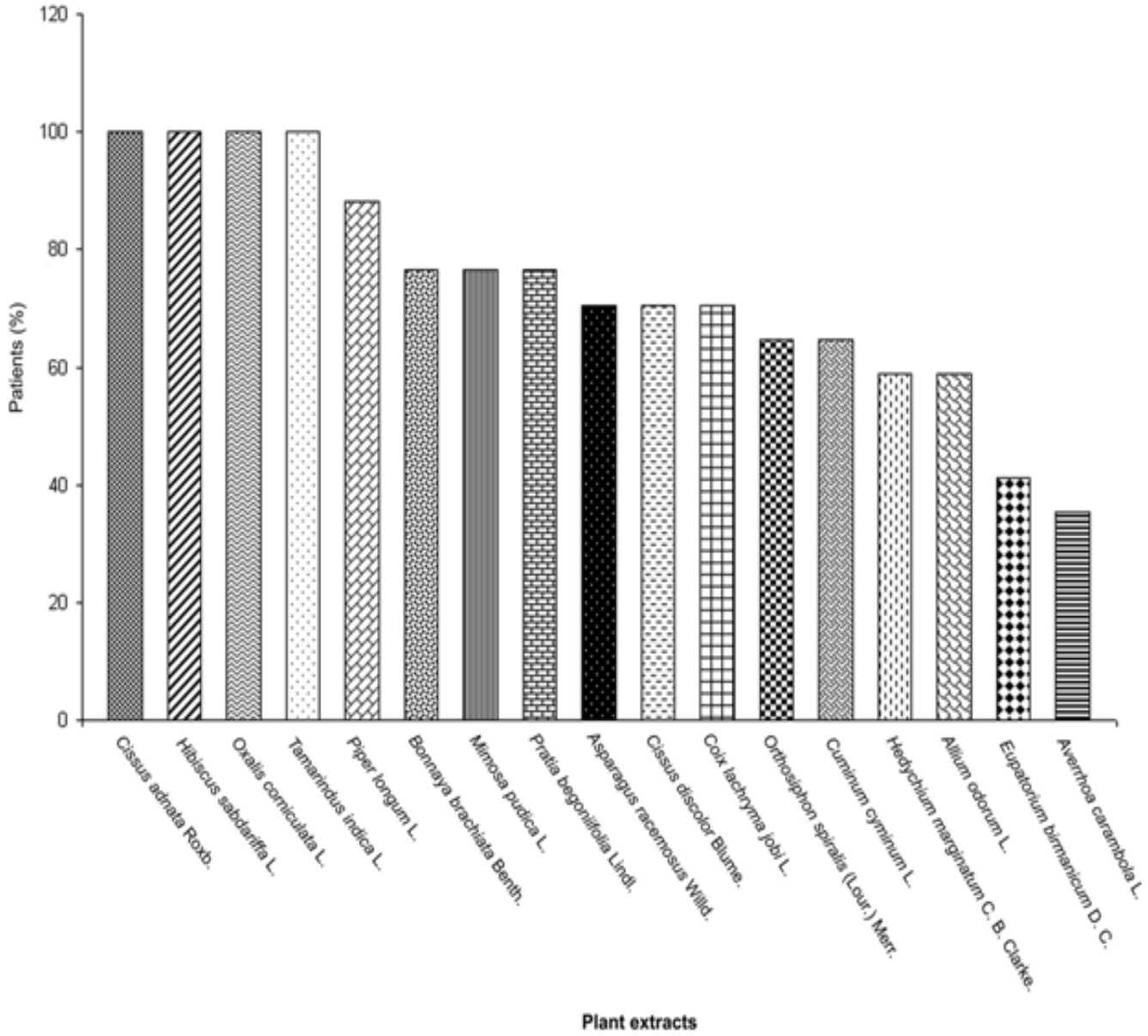
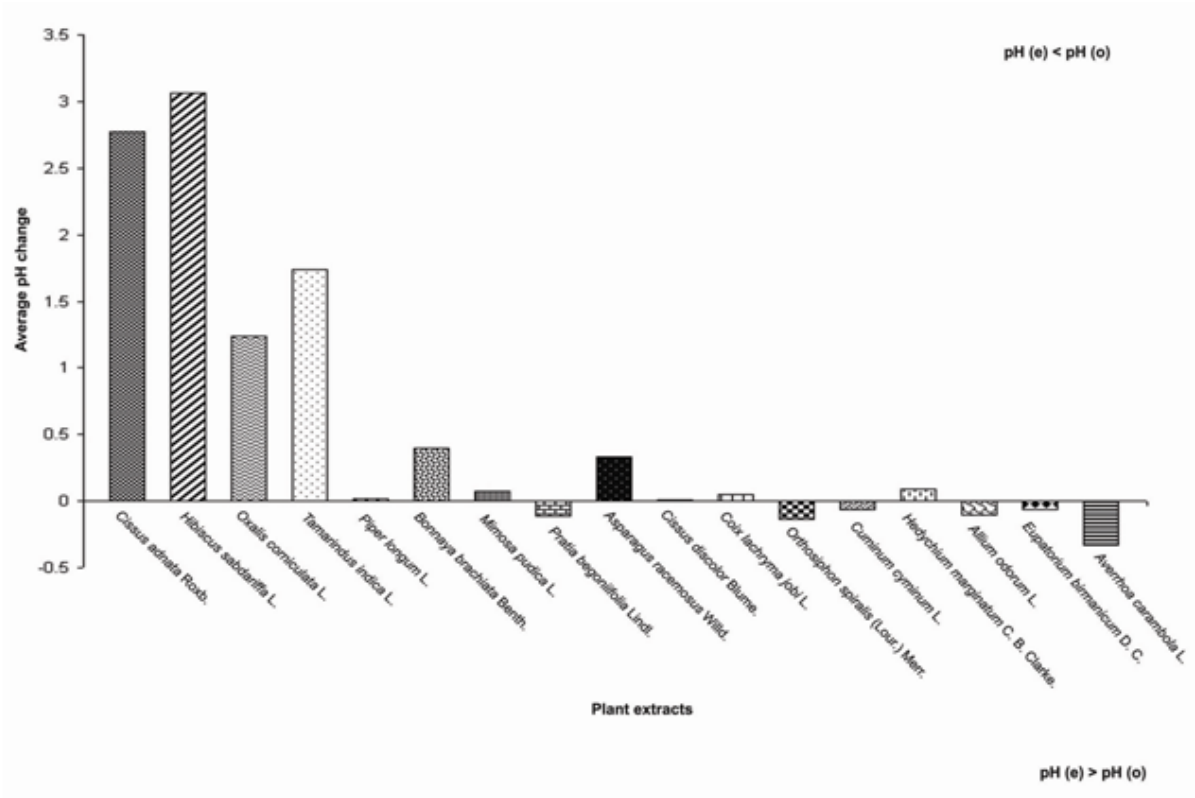


Fig 2: Effect of plant extracts on pH of the urine

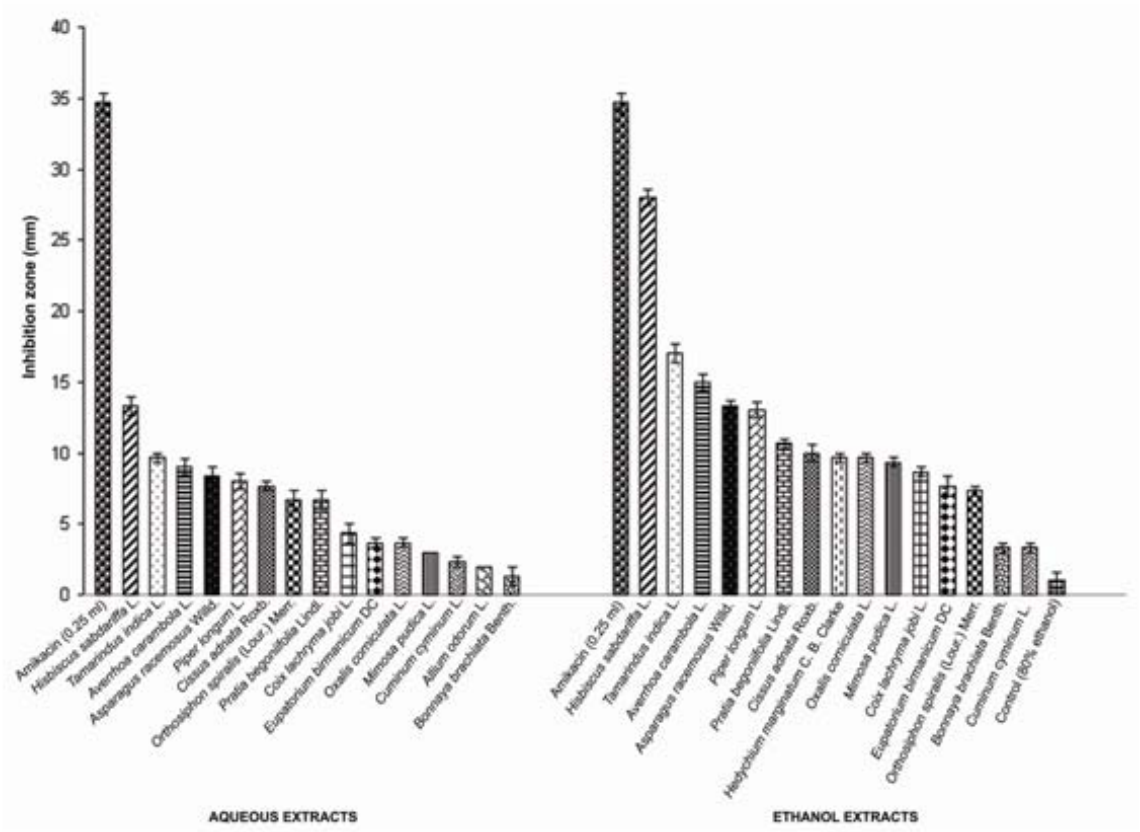


pH (o) – pH of urine without plant extract
pH (e) – pH of urine with plant extract.

Different plant extracts were found to be possessed the capability to alter the pH of the urine, by elevating or decreasing the pH of the urine, rendering to more alkaline or acidic conditions. The plants that could significantly lower the pH of the urine were found to have greater efficacy towards urine decrystallization (Fig 2).

Out of the 17 plants analyzed, both the aqueous and ethanol extracts of *H. sabdariffa*, *T. indica*, *A. carambola*, *A. racemosus* and *P. longum* showed significant effect in controlling the growth of *E. coli* when compared with the antibiotic, Amikacin (0.25 ml) by observing the zones of inhibition most prominently seen in 72 hrs. incubated plates (Fig 3).

Fig 3: Effect of plant extracts on *Escherichia coli*



Aqueous and ethanol extracts of *H. sabdariffa*, *T. indica*, *C. cyminum*, *E. birmanicum* and *C. adnata* were found to show comparatively high anti-bacterial activity against *Proteus mirabilis* as compared to the effect of Amikacin (0.25 ml) (Fig 4).

Fig 4: Effect of plant extracts on *Proteus mirabilis*

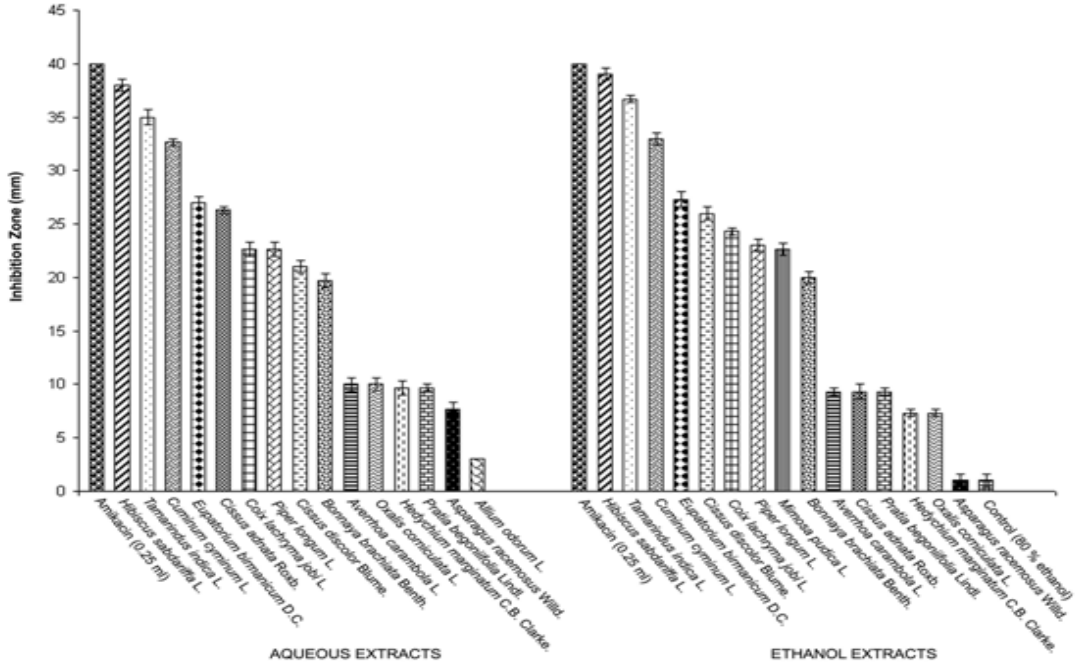
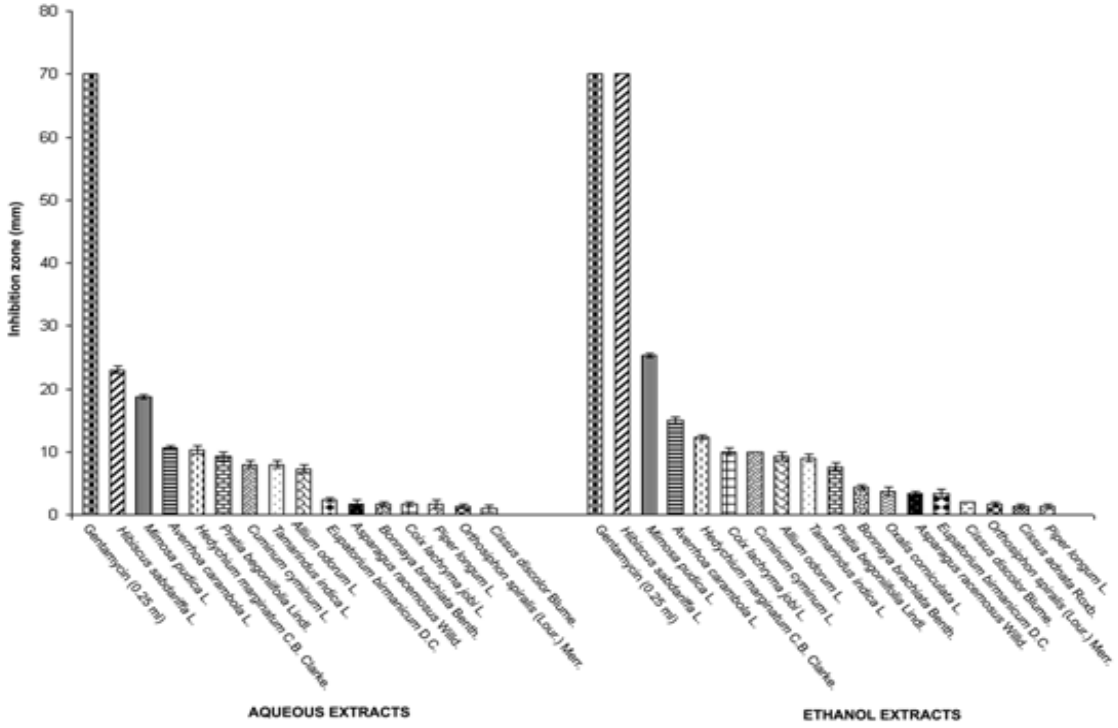


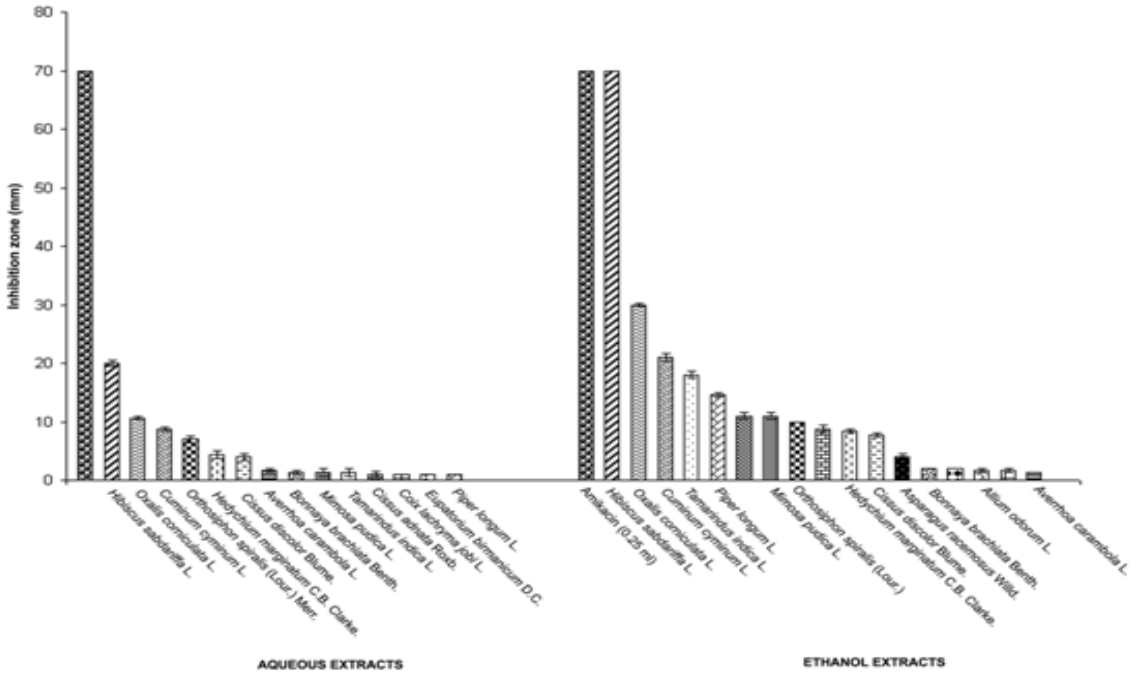
Fig. 5 shows that extracts of *H. sabdariffa*, *M. pudica*, *A. carambola*, *H. marginatum*, *P. begoniifolia*, *C. cyminum* and *T. indica* could comparatively inhibit the growth of *Pseudomonas stutzeri* as compared to that of Gentamycin (0.25 ml).

Fig 5: Effect of plant extracts on *Pseudomonas stutzeri*



Extracts of *H. sabdariffa*, *O. corniculata*, *C. cyminum*, *O. spiralis*, *H. marginatum* exhibited high efficacies in controlling the growth of *K. pneumoniae* sub sp. *pneumoniae* which were at par with 0.25 ml of Amikacin(Fig-6).

Fig 6: Effect of plant extracts on *Klebsiella pneumoniae* sub sp. *pneumoniae*



In almost all the cases, ethanol extracts have higher efficacies than aqueous extracts in controlling the growth of the microbes. Thus, the extracts prepared from dried parts of *C. adnata*, *C. cyminum*, *E. birmanicum*, *H. sabdariffa*, *O. corniculata*, *P. longum* and *T. indica* exhibited a comparative anti-microbial action against the four selected bacteria, viz. *E. coli, P. mirabilis, P. stutzeri* and *K. pneumoniae* sub sp*. pneumoniae*. This finding is in consistency with the results obtained from the extracts prepared from fresh parts of these plants in our earlier experiments (Laikangbam et al. 2009).

Out of the seventeen plants investigated, ethanol extracts of *H. sabdariffa* L., *T. indica* L. and *P. longum* L. showed comparatively higher efficacies than the others at the dose of 1 g/15 mL in dissolving the stone whereas extract of *A. odorum* showed the least efficacy in dissolving the stone (Table 2).

Table 2: Determination of the final weight of the struvite after treatment with different doses of ethanol plant extracts

Sl. No.	Plant samples (80% ethanol extracts)	Final wt. of struvite (1 g/5 mL)[a]	Final wt. of struvite (1 g/10 mL)[b]	Final wt. of struvite (1 g/15 mL)[c]
1	*Allium odorum* L.	0.9997 ± 0.46	0.9984 ± 0.35	0.9981 ± 0.35
2	*Asparagus racemosus* Willd.	0.9597 ± 0.26	0.9357 ± 0.21	0.9231 ± 0.18
3	*Averrhoa carambola* L.	0.9899 ± 0.39	0.9895 ± 0.36	0.9761 ± 0.31
4	*Bonnaya brachiata* Benth.	0.9816 ± 0.35	0.9711 ± 0.32	0.9371 ± 0.20
5	*Cissus adnata* Roxb.	0.9393 ± 0.23	0.9341 ± 0.21	0.9140 ± 0.17
6	*Cissus discolor* Blume.	0.9276 ± 0.20	0.9262 ± 0.20	0.8526 ± 0.14
7	*Coix lachyrma jobi* L.	0.9794 ± 0.32	0.9435 ± 0.23	0.9429 ± 0.25
8	*Cuminum cyminum* L.	0.9989 ± 0.43	0.9764 ± 0.33	0.9667 ± 0.29
9	*Eupatorium birmanicum* DC.	0.9914 ± 0.40	0.9901 ± 0.35	0.9845 ± 0.33
10	*Hedychium marginatum* C.B. Clarke	0.9965 ± 0.44	0.9686 ± 0.29	0.9466 ± 0.27
11	*Hibiscus sabdariffa* L.	0.6206 ± 0.07	0.6202 ± 0.06	0.6090 ± 0.06
12	*Mimosa pudica* L.	0.9671 ± 0.29	0.9485 ± 0.25	0.9406 ± 0.23
13	*Orthosiphon spiralis* (Lour.) Merr.	0.8503 ± 0.12	0.8326 ± 0.16	0.7991 ± 0.12
14	*Oxalis corniculata* L.	0.8854 ± 0.13	0.8267 ± 0.14	0.7942 ± 0.11
15	*Piper longum* L.	0.8936 ± 0.12	0.8490 ± 0.17	0.7241 ± 0.09
16	*Pratia begoniifolia* Lindl.	0.9201 ± 0.16	0.7965 ± 0.12	0.7331 ± 0.10
17	*Tamarindus indica* L.	0.9205 ± 0.17	0.6610 ± 0.09	0.6223 ± 0.07

[a, b, c] Values are expressed as means of three observations ± standard error

Discussion

In India, as in many countries, recent interest has been focused on the therapeutic potential of traditional plants in the context of controlling various diseases by using scientific methods. The role of coliform bacilli in urinary tract infection has long been known in developed countries (Willet and Radojui, 1976; Morton and. Lawande, 1982). Plants remain the most common source of antimicrobial agents.

It has been reported that acidic plant extracts helped in preventing crystal formation of urine, eventually leading to the prevention of kidney stones. In this way, diet and the food habit of the individual

plays an important role in the formation of kidney stones or their prevention. Not only this, pH also has a key role in controlling kidney stone formation as the antibacterial activities of the extracts were found to be increased at an acidic pH. Moreover, phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds that are secondary metabolites of plants serve as defense mechanisms against predation by many microorganisms, insects and herbivores (Lutterodtt et al. 1999; Marjorie, 1999). Increase in the activities of the phyto-constituents in the presence of acidic medium had also been reported (Molan, 1992). Contrary to this, the activity of plant extracts deteriorated considerably at alkaline pH.

Out of the 17 medicinal plants analyzed, *C. adnata*, *H. sabdariffa*, *O. corniculata*, *P. longum* and *T. indica* significantly prevented crystal formation in urine and also help in dissolution of stone. These plants also exhibited strong antibacterial potential. While two other plants *C. cyminum*, *E. birmanicum* also showed significant degree of antimicrobial activity against the four bacterial isolates.

It has been reported that tamarind intake at a dose of 10 gm showed significant beneficial effect in the inhibition of spontaneous crystallization in both normal subjects and in stone formers (Rathore et al. 1993). The present study revealed that *M. pudica* had moderate effect on urine decrystallization, our finding is in agreement with other (Joyamma, 1990), who reported that *M. pudica* was not effective in either preventing stone deposition or dissolving preformed stones. This report suggests the possibility of the involvement of epigenetic factors such as environment, soil nutrients, stress etc. The aqueous extract of *A. racemosus* showed moderate efficacy in preventing crystal formation in urine while the ethanol extract of *A. racemosus* was reported to significantly reduce the elevated level of calculogenic ions in urine and elevated the urinary concentration of magnesium, which is considered as one of the inhibitors of crystallization (Christina et al. 2005).

The demonstration of antimicrobial activities by aqueous extracts provide the scientific basis for the use of these plants in the traditional treatment of diseases, since most traditional medicine systems use water as their solvent in which the decoctions are prepared. In the present investigations, both the aqueous and ethanol extracts are found to be effective against the four bacteria i.e. *P. mirabilis, E. coli, K. pneumoniae* sub sp*. pneumoniae* and *P. stutzeri*. But the ethanol extracts exhibited higher antibacterial activity as compared to the aqueous extract. It has been reported that alcohol is a general solvent and tends to provide a more complete extraction of compounds with a variety of polarities (Evans, 1996). Thus, aqueous extracts may not contain some of the less polar compounds.

Even though stability in pH contributes to the control of urolithiasis, it was observed that not only acidic pH, but alkaline pH also helped in controlling urolithiasis. Medicinal plants such as *C. cyminum* and *E. birmanicum* controls urolithiasis even though they render the urine alkaline. In our present study, since the pre-operative urine samples were collected randomly from different patients, the nature of the stones may be of different types. And this might be one of the contributing factors in influencing varied effects of each plant extract on the urine decrystallization pattern and pH in the patients studied as found in the present investigations. As a result of this, the need of administering different types of medicinal plants to different types of stone i.e. calcium-oxalate stone, struvite, uric acid and urate stones, cystine stone etc. arises to get a promising result. It has been observed that plants which have an acidic pH help in preventing the stone, but struvite is a type of stones formed due to urinary tract infection in alkaline pH**.** It is still a general assumption that the plant extracts prepared through local traditional medicine systems have some positive effect on urolithiasis**.** Thus, control of urolithiasis largely depends upon the bioactive molecules present in the medicinal plants. At this juncture, attention is needed for carrying out chemical and ethno-pharmacological studies as a control and preventive measure for urolithiasis. Such investigations may lead to the discovery of novel bioactive molecules and several works are undertaking to identify the compounds responsible for this biological activity.

Conclusion

Biological evaluation of potential medicinal plants such as *C. adnata*, *C. cyminum*, *E. birmanicum*, *H. sabdariffa*, *O. corniculata*, *P. longum* and *T. indica* demonstrates promising results on urine decrystallization, antibacterial activity and dissolution of stone thereby serving as controlling agents of urolithiasis and which eventually would lead to a break-through in the prevention of urolithiasis. Thus, this investigation would further open up new avenues to the use of these medicinal plants in drug development for the treatment of urolithiasis.

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