



## Anti-bacterial efficacy of elite medicinal plants on urolithiasis inducing flora

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### Abstract

Medicinal plants are valuable sources of novel antibacterials which are associated with the prevention and control of urolithiasis. Seventeen plant species, namely *Allium odorum* (Linnaeus), *Asparagus racemosus* (Willdenow), *Averrhoa carambola* (Linnaeus), *Bonnaya brachiata* (Benth), *Cissus adnata* (Roxburgh), *Cissus discolor* (Blume), *Coix lachryma jobi* (Linnaeus), *Cuminum cyminum* (Linnaeus), *Eupatorium birmanicum* (De Candolle), *Hedychium marginatum* (Charles Baron Clarke), *Hibiscus sabdariffa* (Linnaeus), *Mimosa pudica* (Linnaeus), *Orthosiphon spiralis* (Merrill), *Oxalis corniculata* (Linnaeus), *Piper longum* (Linnaeus), *Pratia begoniifolia* (Lindley) and *Tamarindus indica* (Linnaeus), were screened for potential antibacterial activity against four selected urolithiasis inducing flora, namely *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas stutzeri* and *Klebsiella pneumoniae* subsp. *pneumoniae*. This study was based on antibacterial susceptibility test in which the antibacterial activities of aqueous and ethanol extracts of the medicinal plants were determined by standard disc-diffusion technique. Observations were noted at the end of 24, 48 and 72 h incubation. From the screening experiments, *H. sabdariffa* exhibited the highest antibacterial activity in almost all the test organisms. Others, namely *C. adnata*, *C. cyminum*, *O. corniculata*, *P. longum* and *T. indica* also exhibited significant antibacterial effect to a certain degree. Ethanol extracts showed more efficacy for almost all the plants studied. Thus, *C. adnata*, *C. cyminum*, *H. sabdariffa*, *O. corniculata*, *P. longum* and *T. indica* show promising roles in the prevention and cure of urolithiasis. This study shows the potential and healing powers of medicinal plants and will be a great boon to the human society. In fact, folk or traditional medicinal uses represent 'leads' that could shortcut the discovery of modern medicines.

**Key words:** Medicinal plants, urolithiasis, antibacterial activity, aqueous extracts, ethanol extracts, inhibition zone.

### Introduction

One of the most common problems faced by the human society is the stone-case, out of which urolithiasis is one of them. Urolithiasis is synonymous to calculus formation at any level in the urinary collecting system but most often, calculus arises in the kidney <sup>1</sup>. It is one of the commonest renal disorders in Manipur. It occurs more frequently in man than woman but rare in children <sup>2</sup>. Recurrent stone formation is probably the most important problem in the prognosis of patients who have undergone operations for renal and ureteric calculi. The etiology is complex and is considered multifactorial, such as food habit, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque, microliths, urinary tract infection etc. <sup>3</sup>.

When the urinary tract is infected by urea-splitting organisms, the urea which is being excreted in the urine splits resulting to the formation of ammonia as a by-product which renders the urine alkaline. In this alkaline urine, there tends to be precipitated crystals of calcium and magnesium phosphate and calcium carbonate which are present in large amount in that medium. There is a strong tendency to the formation of calcium phosphate and calcium carbonate calculi when abundance crystalloids are found continuously in the medium <sup>4</sup>. Another mechanism by which bacterial infection may induce stone formation is by crystal adherence.

Thomson and Stamey <sup>5</sup> have confirmed the fact that struvite and other urinary calculi are caused by the action of bacteria on urine. They also contain numerous infectious bacteria within the structure. The majority of urea-splitting organisms are of the species *Proteus*. On the other hand, organisms such as *Pseudomonas*, *Klebsiella*, *Staphylococcus*, occasionally *E. coli* and even *Mycoplasma* are capable of producing bacterial urease <sup>6,7</sup>. Robertson <sup>8</sup> observed that infected stones were associated with the organisms like *E. coli*, *Proteus* sp., *Klebsiella* sp., *Streptococcus*, *Staphylococcus*, *Pseudomonas* and *Ureaplasma urealyticum*. Griffith and Osborne <sup>9</sup> have shown that the end products of urealys damage the glycosaminoglycan layer of the renal urothelial cells leading to the bacterial adherence, followed by biofilm formation and mineral encrustation. So, a careful microbiological investigation to find and treat the infection responsible for the stone formation is mandatory.

Medicinal plants are the local heritage with global importance. Out of the total 422,000 flowering plants reported from the world <sup>10</sup>, more than 50,000 are used for medicinal purposes <sup>11</sup>. Medicinal plants have been the subjects of man's curiosity since times immemorial <sup>12</sup>. Almost every civilization has a history of medicinal plant use <sup>13</sup>. Utilization of plants for medicinal purposes in India has been documented long back in ancient Hindu scriptures like *Rigveda* (4500-1600 B.C.), *Charak Samhita* (1000-

800 B.C.), *Sushrut Samhita* (800-700 B.C.) and others. However, organized studies in this direction were initiated in 1956<sup>14</sup> and of late, such studies are gaining recognition and popularity due to loss of traditional knowledge and declining plant population. Therefore, the use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health, has been widely observed<sup>15</sup>.

Manipur (24°49' N and 93°52' E) is a state embedded with very rich biotic resources representing a mixed flora and fauna of the Himalayan region and Malayan Archipelago. Manipur, which is a part of North East India, happens to be within the Indo-Burmese mega-biodiversity hot spot. This region is one of the eight hottest hotspots in terms of richness of endemic species diversity of both plants and vertebrates and high degree of threat<sup>16</sup>. Many varieties of plants including those used by traditional medical practitioners grow luxuriantly in this region. Ayurveda is an ancient Indian form of medicine which deals with plants and plant extracts. This indigenous form of medicine uses the active ingredients present in plants for treating diseases<sup>17</sup>. Medicinal plants have curative properties due to the presence of various complex chemical substances of different composition, which are generally secondary plant metabolites found in one or more parts of these plants. Many traditional medicinal plants have been identified as cures, retardants or sustenance of various health ailments. Interest in phytomedicine has exploded during the last few years. About 500 different plant species are used as key ingredients and many are still being collected from the wild<sup>18</sup>. Phytochemicals are frequently considered to be less toxic and more free from side effects than synthetic ones<sup>19</sup>.

Scientists throughout the world are trying to explore the precious assets of medicinal plants to help the suffering human population. Furthermore, in the world, more than 30% of the pharmaceutical preparations are herbal based<sup>20</sup>. Ethnobotanical studies have brought light to numerous plants having significant medicinal properties which were earlier unknown to the scientific world<sup>21-25</sup>. The World Health Organization (WHO) estimates that about 80% of the world's population relies mainly on herbal medicine for primary healthcare and is reported to have minimal side effects and about 85% of traditional medicine involves the use of plant extracts<sup>26-30</sup>. However, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants.

In India, as in many countries, recent interest has focused on the therapeutic potential of traditional plants in the context of various diseases by using scientific methods. The role of coliform bacilli in urinary tract infection has long been known in developed countries<sup>31,32</sup>. The introduction of antibiotics for the chemotherapy of bacterial infections has been one of the most important medical achievements of the past 50 years. However, the emergence of bacterial resistance to antibiotics undermines the therapeutic utility of existing agents, creating a requirement for the discovery of new antibacterial drugs<sup>33</sup>. The rising incidence in multi-drug resistance (MDR) amongst pathogenic microbes has further necessitated the need to search for newer antibiotic source. Plants remain the most common source of antimicrobial agents. Many of the existing synthetic drugs cause various side effects. Hence, drug development from plant-based compounds could be useful in meeting this demand for newer drugs with minimal side effects.

Scientific investigations on the indigenous medicines prepared from plant products used by the Tribals and Meiteis of Manipur may prove to be of great pharmacological importance leading to the advent of newer drugs, which could be at par with the modern allopathic medicines in terms of efficacy. The aim of the study was to screen medicinal plant species grown in Manipur for potential antibacterial activity against urolithiasis inducing flora.

## Materials and Methods

**Collection of plant samples:** Fresh plants/plant parts were collected from various places of Imphal-west district (24°37'N and 93°30'E), Manipur, India. The plant samples (Table 1) were collected and deposited in the Herbarium of Manipur University, Imphal, and respective voucher numbers were assigned. Fresh plant samples were washed under running tap water and air-dried for about 10 minutes.

**Crude extraction of plant samples:** Aqueous extracts of the plant samples were prepared by crushing the fresh plants with the help of a mortar and pestle in the ratio of 0.1 g ml<sup>-1</sup> of distilled water. The plant extract thus obtained was filtered through Whatmann No.1 filter paper, sterilized and stored at refrigerated conditions for future use.

Thirty grams of the plant material was extracted in 300 ml of 80% ethanol using a mortar and pestle. The plant extract thus obtained was filtered through Whatmann No.1 filter paper, sterilized and stored in airtight bottles at 4°C for future use.

**Collection of urine samples:** The present study was performed on pre-operative urine. First voided morning mid-stream urine samples were collected aseptically from 25 urolithiatic patients admitted in the Urology Department, Regional Institute of Medical Sciences, Imphal, in sterile wide-mouth containers by giving proper guidelines for urine collection for both males and females. The subjects were chosen randomly consisting of mostly adults (aged 20-80), out of which 14 were males and 11 were females. Relevant information about the disease was collected from each patient after taking prior consent from the patient.

**Micro-organisms:** In human beings, urinary tract infections are mostly caused by Gram-negative bacteria and rarely by Gram-positive bacteria. Microbial strains *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas stutzeri* and *Klebsiella pneumoniae* subsp. *pneumoniae* were selected for the present study. All the test organisms were clinical isolates obtained from the urine samples of infected patients. These bacterial strains are potential human pathogens. Isolation and identification of the test organisms were performed as described by Cruickshank *et al.*<sup>34,35</sup>. The bacterial isolates were sub-cultured periodically on blood-agar plates and prepared for the assessment of plant extract activity.

**Antibacterial assay:** The antibacterial assay was carried out by using standard disc-diffusion technique. The *in vitro* antimicrobial activity was performed against overnight grown cultures of four selected bacteria, namely *P. mirabilis*, *E. coli*, *P. stutzeri* and *K. pneumoniae* subsp. *pneumoniae* on blood-agar media. This can be achieved by first thoroughly spreading the overnight grown bacteria (1 O.D.) on blood-agar plates. Then, the sterile filter discs were placed onto the inoculated plates. Twenty µg of

**Table 1.** Tested plant species, their voucher numbers, families and local names.

Plant species (Voucher No.)	Family	Local name (Manipuri)
<i>Allium odorum</i>	Liliaceae	Maroi-nakuppi
<i>Asparagus racemosus</i> (Deb 253)	Liliaceae	Nunggarei-angouba
<i>Averrhoa carambola</i> (Deb 2210)	Averrhoaceae	Heinajom
<i>Bonnaya brachiata</i>	Scrophulariaceae	Keehomann
<i>Cissus adnata</i> (Deb 573)	Vitaceae	Kongouyen
<i>Cissus discolor</i> (Deb 450, 482 & 543)	Vitaceae	Kongouyen-laba
<i>Coix lachryma jobi</i> (Mukherjee 3522)	Poaceae	Chaning
<i>Cuminum cyminum</i> (Deb 2341)	Apiaceae	Jeera
<i>Eupatorium birmanicum</i>	Asteraceae	Langthrei
<i>Hedychium marginatum</i> (Deb 782 & 832)	Zingiberaceae	Takhellei-angangba
<i>Hibiscus sabdariffa</i> (Deb 1377)	Malvaceae	Silot-sougree
<i>Mimosa pudica</i> (Deb 2467)	Leguminosae	Kangphal-ikaithabi
<i>Orthosiphon spiralis</i> (Kanjilal 502)	Lamiaceae	Leikhamann
<i>Oxalis corniculata</i> (Mukherjee 2808)	Oxalidaceae	Meitei Yensil
<i>Piper longum</i> (Deb 1265)	Piperaceae	Taboppi
<i>Pratia begoniifolia</i>	Campanulaceae	Nungai-peruk
<i>Tamarindus indica</i> (Deb 2477)	Leguminosae	Mangge

each plant extract (both aqueous and alcoholic) was added onto the discs. After addition of each of the plant extracts, the inoculated plates were kept at room temperature for about 1 hour to enable diffusion of the plant extracts and incubated at 37°C. Microbial growth inhibition was determined by measuring the diameter of the zone of inhibition which was assessed at 24, 48 and 72 h incubation.

### Results and Discussion

Successful prediction of bioactive compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as the solvent but in our studies, we found that plant extracts in organic solvent (ethanol) provided more consistent anti-microbial activity compared to those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the medium used in the assay. The results of screening are presented in Figs 1-4. The aqueous and ethanol extracts of seventeen plant species belonging to fourteen different families were tested against four Gram-negative bacteria species using standard disc-diffusion technique.

Out of the 17 medicinal plant species analyzed, Fig. 1 projects that both the aqueous and ethanol extracts of *C. adnata* showed the highest efficacy of anti-bacterial activity against *P. mirabilis*. The aqueous and ethanol extracts of *C. cyminum*, *H. sabdariffa*, *T. indica* and *P. longum* also showed comparative efficacy against the microbe. *P. mirabilis* was resistant to the aqueous extracts of *A. racemosus*, *E. birmanicum* and *O. spiralis* while their ethanol extracts were found to inhibit the organism. This shows that

ethanol extracts exhibited higher antibacterial activity. This finding is in consistence with the report of Evans<sup>36</sup> that alcohol is a general solvent which tends to provide a more complete extraction of compounds with a variety of polarities and the aqueous extracts may not contain some of the less polar compounds. It was also reported that the ethanol extract of *A. racemosus* significantly reduced the elevated level of calculogenic ions present in urine and it elevated the urinary concentration of magnesium, which is considered as one of the inhibitors of crystallization<sup>37</sup>.

Both the aqueous and ethanol extracts of *H. sabdariffa* exhibited the highest antibacterial activity against *E. coli*. The aqueous and ethanol extracts of *C. adnata*, *O. corniculata*, *T. indica* and *P. longum* had also inhibitory effect on *E. coli* unlike *C. discolor* which showed least inhibitory effect (Fig. 2). Rathore *et al.*<sup>38</sup> reported that tamarind intake at a dose of 10 g showed significant beneficial effect in the inhibition of spontaneous crystallization in both normal subjects and in stone formers.

The extracts (aqueous and ethanol) of *H. sabdariffa* showed the highest potential in inhibiting the growth of *K. pneumoniae* subsp. *pneumoniae*, followed by *O. corniculata*. The extracts of *A. carambola*, *C. adnata*, *C. cyminum*, *H. marginatum*, *P. longum* and *T. indica* showed higher efficacy in alcoholic state in controlling the growth of the microbe. The ethanol extracts of *C. discolor* and *E. birmanicum* couldn't inhibit the growth of the microorganism (Fig. 3).

Aqueous and ethanol extracts of *H. sabdariffa* significantly inhibited the growth of *P. stutzeri*. The plants that exhibited antibacterial activity to a certain degree were *A. carambola*, *C. adnata*, *H. marginatum*, *M. pudica*, *O. corniculata*, *P. longum* and *T. indica* (Fig. 4). Both the aqueous and ethanol extracts of *A. odorum* couldn't inhibit the growth of the microbe. The aqueous

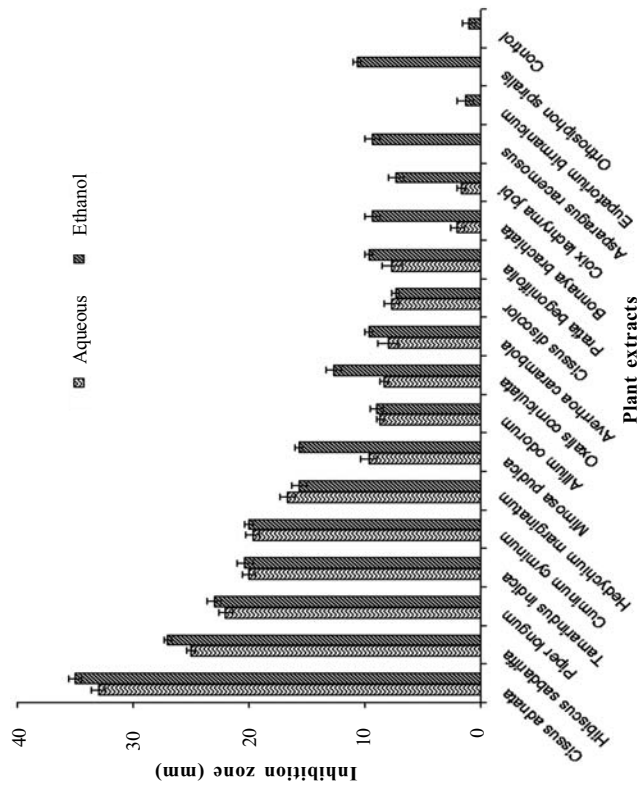


Figure 1. Effect of plant extracts on *Proteus mirabilis*.

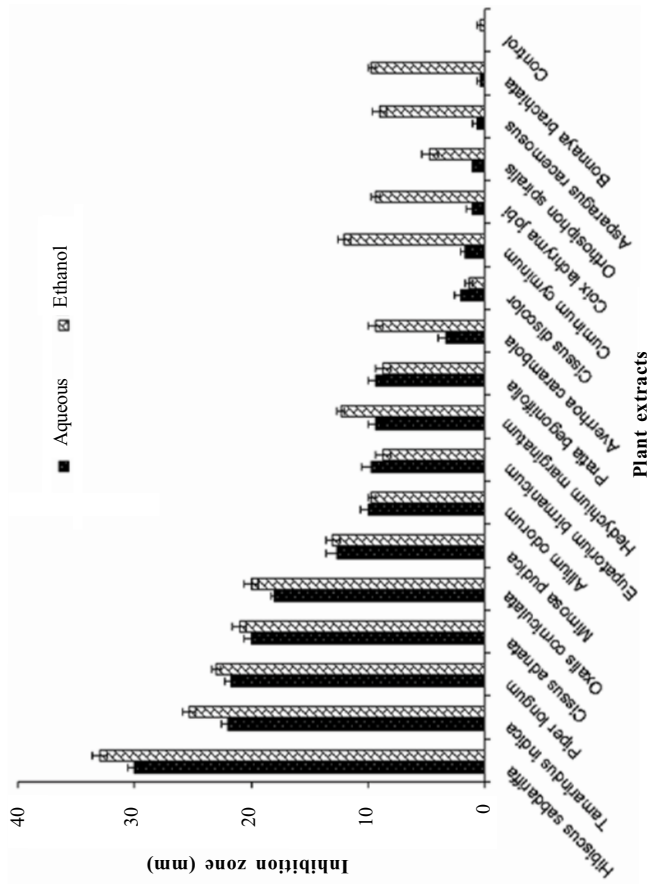


Figure 2. Effect of plant extracts on *Escherichia coli*.

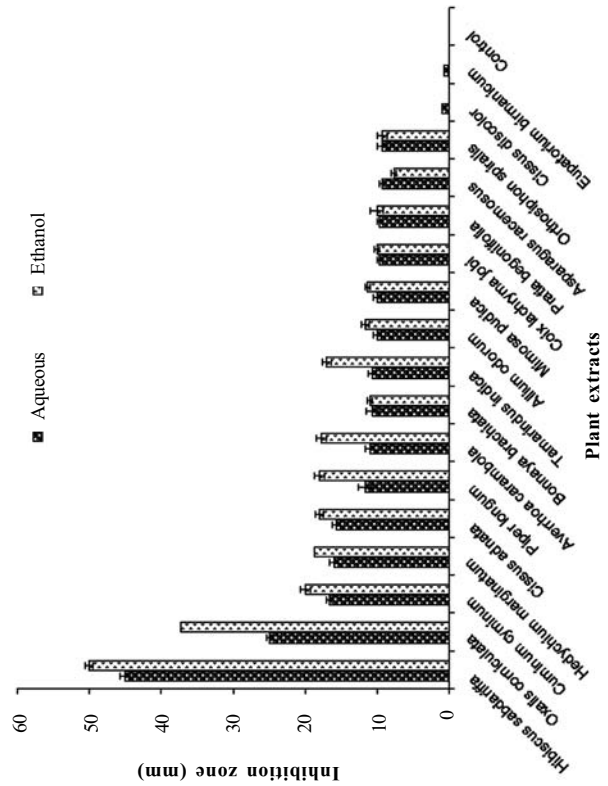


Figure 3. Effect of plant extracts on *Klebsiella pneumoniae* subsp. *pneumoniae*.

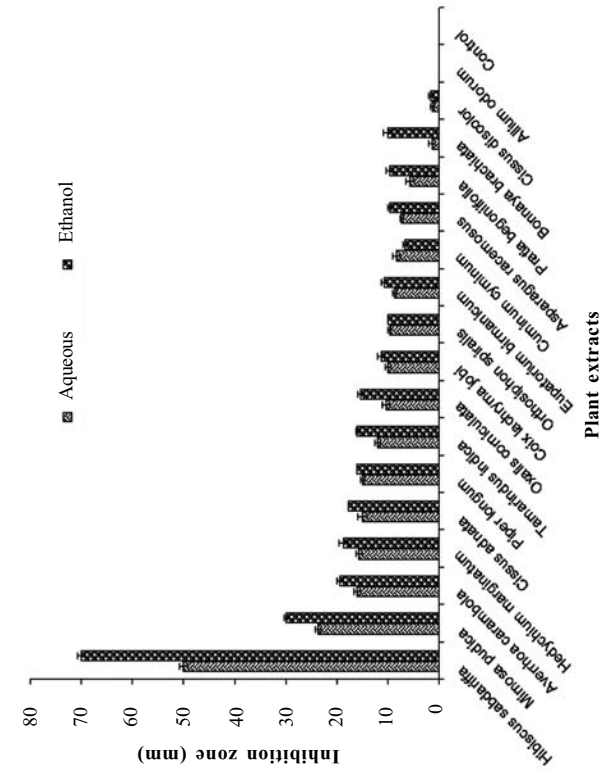


Figure 4. Effect of plant extracts on *Pseudomonas stutzeri*.

extracts of *B. brachiata* and *C. discolor* showed least efficacy against the microbe.

Thus, the observations conferred that both the aqueous and ethanol extracts of *H. sabdariffa* showed the highest antibacterial activity in almost all the test organisms. Others, namely *C. adnata*, *C. cyminum*, *O. corniculata*, *P. longum* and *T. indica* also exhibited significant antibacterial effect to a certain degree. From our investigation of screening different plant species, the results obtained confirmed the therapeutic potency of some plants used in traditional medicine.

The demonstration of antimicrobial activity by aqueous extracts provides the scientific basis for the use of these plants in the traditional treatment of diseases, since most traditional medicine systems employ water as their solvent in which the decoctions are prepared. Both the aqueous and ethanol extracts were effective against the four bacteria, namely *P. mirabilis*, *E. coli*, *K. pneumoniae* subsp. *pneumoniae* and *P. stutzeri* in which ethanol extracts exhibited a higher degree of antibacterial activity.

The results of the present study form a good basis for selection of candidate plant species for further phytochemical and pharmacological investigation. The findings also support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It has been reported that phytochemical constituents such as tannins, flavonoids, alkaloids and several other aromatic compounds that are secondary metabolite of plants served as defense mechanisms against predation by many microorganisms, insects and herbivores<sup>39,40</sup>. Thus, the most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation. Such investigations may lead to the discovery of novel bioactive molecules. Extensive work is in progress to identify compounds responsible for this biological activity.

### Conclusions

Conclusion can be drawn that the medicinal plants viz., *C. adnata*, *C. cyminum*, *H. sabdariffa*, *O. corniculata*, *P. longum* and *T. indica* could be employed for the eradication of bacterial flora associated with urolithiasis with reference to promising results obtained on antibacterial activity against the four bacterial isolates. This eventually would lead to a break-through in the prevention of urolithiasis. The exploitation of these potential herbs for the cause of urolithiasis prevention and cure would help in subsiding the existing problem of kidney stone formation up to an appreciable degree. 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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### References

- <sup>1</sup>Kumar, V., Cotran, R.S. and Robin, S.L. 1992. Basic Pathology. 5<sup>th</sup> edn. Prism Book Pvt. Ltd., Bangalore, India, pp. 347-471.
- <sup>2</sup>Smith, D.R. 1978. General Urology. 9<sup>th</sup> edn. Lange Medical Publications, California, pp. 212-232.
- <sup>3</sup>Fowler, C. 1995. The kidneys and ureters. In Mann, C.V., Russel, R.C.G. and Williams, N.S. (eds). Bailey and Love's Short Practice of Surgery. 22<sup>nd</sup> edn. ELBS, Chapman and Hall, London, pp. 915-939.
- <sup>4</sup>Chute, R. and Suby, H.I. 1943. Prevalence and importance of urea splitting bacterial infections of the urinary tract in the formation of calculi. *J. Urol.* **44**:590-595.
- <sup>5</sup>Thompson, R.B. and Stamey, T.A. 1973. Bacteriology of infected stones. *Urology* **2**:627-633.
- <sup>6</sup>Friedlander, A.M. and Braude, A.I. 1974. Production of bladder stone by human T microplasmas. *Nature* **247**(5435):67-69.
- <sup>7</sup>Griffith, D.P., Musher, D.M. and Itin, C. 1976. Urease: The primary cause of infection-induced urinary stones. *Invest. Urol.* **13**:346-350.
- <sup>8</sup>Robertson, W.G. 1992. Aetiological factors in stone formation. In Cameron, S., Davison, A.M., Grunfeld, J.P., Kerr, D. and Ritz, E. (eds). Oxford Textbook of Clinical Nephrology. Vol. 3. Oxford Medical Publication, pp. 1846-1882.
- <sup>9</sup>Griffith, D.P. and Osborne, C.A. 1987. Infection (Urease stone). *Miner. Electrolyte Metal.* **13**:278-285.
- <sup>10</sup>Govaerts, R. 2001. How many species of seed plants are there? *Taxon* **50**:1085-1090.
- <sup>11</sup>Schippmann, U., Leaman, D.J. and Cunningham, A.B. 2002. Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues. In FAO. Biodiversity and the ecosystem approach in agriculture, forestry and fisheries. Satellite Event on the Occasion of the Ninth Regular Session of the Commission on Genetic Resources for Food And Agriculture. Rome 12-13 October 2002. Interdepartmental Working Group on Biological Diversity for Food and Agriculture.
- <sup>12</sup>Constable, F. 1990. Medicinal plant biotechnology. *Planta Med.* **56**:421-425.
- <sup>13</sup>Ensminger, A.H., Ensminger, M.E., Kolande, J.E. and Robson, J.R.K. 1983. Food and Nutrition Encyclopedia. Vol. 2. Pegus Press, California, U.S.A. pp. 1427-1441.
- <sup>14</sup>Rao, R.R. 1996. Traditional knowledge and sustainable development key role of ethnobiologists. *Ethnobotany* **8**:14-24.
- <sup>15</sup>Edward, A. 2001. Pathogenesis *Justicia adhatoda*. New, Old and Forgotten Remedies, pp. 210-220.
- <sup>16</sup>Myers, N., Mittermeier, R.A., Mittermeier, C.G., Fonseca, G.A.B. and Kent, J. 2000. Biodiversity hotspots for conservation priorities. *Nature* **403**:853-858.
- <sup>17</sup>Lewis, H.S. and Elvin-Lewis, M.P.H. 1977. Medical Botany. Wiley, New York, pp. 217-218.
- <sup>18</sup>Mendelsohn, R. and Balick, M. 1994. The value of undiscovered pharmaceuticals in tropical forests. *Econ. Bot.* **49**(2):223-228.
- <sup>19</sup>Momin, A. 1987. Role of indigenous medicine in primary health care. Proceedings of First International Seminar on Unani Medicine, New Delhi, India, p. 54.
- <sup>20</sup>Shinwari, M.I. and Khan, M.A. 1998. Indigenous use of medicinal trees and shrubs of Margalla Hills National Park, Islamabad. *Pak. J. Forest.* **48**(1-4):63-90.
- <sup>21</sup>Pushpangadan, P., Rajasekharan, S., Kumar, R., Jawahar, C.R., Velayudhan Nair, V., Lakshmi, N. and Sarad Amma, L. 1988. Arogyapacha (*Trichopus zeylanicus*) - the Ginseng of Kani tribes of Agasthyar Hills (Kerala) for evergreen health and vitality. *Ancient Science of Life* **7**:13-16.
- <sup>22</sup>Pushpangadan, P., Rajasekharan, S., Latha, P.G., Evans, D.A. and Valsaraj, R. 1995. Further studies on the pharmacology of *Trichopus zeylanicus*. *Ancient Science of Life* **14**:127-135.
- <sup>23</sup>Kaul, M.K., Sharma, P.K. and Singh, V. 1989. Ethnobotanical studies in north west and trans-Himalaya VI. Contribution to the ethnobotany of Basohli-Bani region. *J & K. Bulletin of Botanical Survey of India* **31**:89-94.

- <sup>24</sup>Subramoniam, A., Madhavachandran, V., Rajasekharan, S. and Pushpangadan, P. 1997. Aphrodisiac property of *Trichopus zeylanicus* extract in male mice. *J. Ethnopharmacol.* **57**:21-27.
- <sup>25</sup>Subramoniam, A., Evans, D.A., Valsaraj, R., Rajasekharan, S. and Pushpangadan, P. 1999. Inhibition of antigen-induced degranulation of sensitized mast cells by *Trichopus zeylanicus* in mice and rats. *J. Ethnopharmacol.* **68**:137-143.
- <sup>26</sup>Vieira, R.F. and Skorupa, L.A. 1993. Brazilian medicinal plants gene bank. *Acta Hort.* **330**:51-58.
- <sup>27</sup>Farnsworth, N.R. 1994. Ethnopharmacology and drug development. *Ethnobotany and the Search for New Drugs*. Ciba Foundation Symposium 185. John Wiley and Sons, Chichester, pp. 42-51.
- <sup>28</sup>Bibitha, B., Jisha, V.K., Salitha, C.V., Mohan, S. and Valsa, A.K. 2002. Antibacterial activity of different plant extracts. Short Communication. *Indian J. Microbiol.* **42**:361-363.
- <sup>29</sup>Maghrani, M., Zeggwah, N., Michel, J. and Eddoules, M. 2005. Antihypertensive effect of *Lepidium sativum* in spontaneously hypertensive rats. *J. Ethnopharmacol.* **102**(1-2):193-197.
- <sup>30</sup>Mukherjee, P.K. and Wahil, A. 2006. Integrated approaches towards drug development from Ayurveda and other systems of medicine. *J. Ethnopharmacol.* **103**:25-35.
- <sup>31</sup>Willet, W.C. and Radojui, V. 1976. Urinary tract pathogen and antibiotic sensitivity patterns in Dares Salaam. *East Afr. Med. J.* **53**:685-692.
- <sup>32</sup>Morton, R.E. and Lawande, R.I.I. 1982. Frequency of clinical features of urinary tract infection in pediatric out patients. *Ann. Trop. Paediatr.* **2**:113.
- <sup>33</sup>Chopra, I., Hesse, L. and O'Neill, A.J. 2002. Exploiting current understanding of antibiotic action for discovery of new drugs. *J. Appl. Microbiol.* **92**(Suppl.):4S-15S.
- <sup>34</sup>Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. 1975. *Medical Microbiology*. Vol. 2. 12<sup>th</sup> edn. Longman Group Ltd., Britain, pp. 96-208.
- <sup>35</sup>Cruickshank, R., Duguid, J.P., Marmion, B.P. and Swain, R.H.A. 1975. *Medical Microbiology*. Vol. 2. 12<sup>th</sup> edn. Longman Group Ltd., Britain, pp. 356-439.
- <sup>36</sup>Evans, W.C. 1996. *Trease and Evans' Pharmacognosy*. 14<sup>th</sup> edn. W.B. Saunders, London.
- <sup>37</sup>Christina, A.J., Ashok, K., Packialakshmi, M., Tobin, G.C., Preethi, J. and Muruges, N. 2005. Antilithiatic effect of *Asparagus racemosus* Willd. on ethylene glycol-induced lithiasis in male albino Wistar rats. *Methods Find. Exp. Clin. Pharmacol.* **27**(9):633-638.
- <sup>38</sup>Rathore, P., Pendse, A.K., Handa, S., Sharma, K. and Singh, P.P. 1993. Effectiveness of tamarind (*Tamarindus indicus*) therapy (3 gm and 10 gm) on calcium oxalate and calcium phosphate crystallization using three different methods. *Indian J. Clin. Biochem.* **8**:136-143.
- <sup>39</sup>Lutterodt, G.D., Ismail, A., Basheer, R.H. and Baharudin, H.M. 1999. Antimicrobial effects of *Psidium guajava* extract as one mechanism of its antidiarrhoeal action. *Malays. J. Med. Sci.* **6**(2):17-20.
- <sup>40</sup>Cowan, M.M. 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* **12**(4):564-582.