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Mini Review

Urolithiasis: Phytotherapy as an adjunct therapy

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Role of herbal drugs and medicinal plant extracts in the successful treatment of urolithiasis, classified as the third most common urinary tract diseases is well documented. Ayurvedic plants and their components mediate antilithogenic effects by altering ionic composition of urine, being diuretic, antioxidant or having antimicrobial activity. Therapeutic peptides and proteins have unique place in pharmaceutical biotechnology due to their critical roles in cell biology. The innovation in antilithiatic proteins is that they are anionic, rich in acidic amino acids which make oxalate unavailable by interacting with calcium and have EF Hand domain which is a characteristic feature of various calcium binding protein like calgranulin, osteopontin. The review provides a background on the pathogenesis of urolithiasis and medical treatments. It focusses on the present research evaluating the scientific basis of antilithiatic potential of various plants and role of plant proteins as therapeutic agents thus opening new vista in the management of urolithiasis. Further investigations are required to fully decipher the mode of action of the potent biomolecules so as to exploit their preventive and therapeutic potential.

Keywords: Phytoconstituents, Phytotherapy, Urolithiasis

Humankind is known to be afflicted by urinary stone diseases first reported in Egyptian mummies dated 4000 BC and reference being made in early Sanskrit documents in India between 3000 and 2000 BC[1]. In the light of these historical clues, it appears that mankind is afflicted by urinary stones since antiquity. Depending on the socio-economic conditions and subsequent changes in the dietary habits, the overall probability of stone formers differs in various part of world: 5-9% Europe, 12% Canada, 13-15% USA and 20% Saudi Arabia. The "stone belts" of the world are located in the countries of the Middle East, North Africa, Mediterranean regions, North-western states of India and Southern states of USA[2]. In India, with a prevalence rate of 15%[3], two high incidence stone belts have been found to occur. The first belt starts from Amritsar in North and while passing through Delhi and Agra ends up in U.P. The other belt which starts from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been in West Bengal and coastal areas of Maharashtra, Karnataka, Kerala, Tamil Nadu and Andhra Pradesh[4]. In the last few years, incidence of urinary stones is increasing with a decrease in age of onset. With a high prevalence rate of 2-20%[5], an expected recurrence rate of ~ 50% and economic burden due to costly surgical treatment, stone disease has an important effect on health care system[6]. There has been a continuous increase in the kidney stone cases reported in the wake of global warming. Researchers predict that by 2050, higher temperatures will cause an additional 1.6 million to 2.2 million kidney-stone cases, representing up to a 30% growth in some areas[7]. The present review provides a general background about pathophysiology of renal stone disease, medical treatment including dietary recommendations, drugs and surgical interventions. It focuses on data highlighting the present trends in research of medicinal plants in form of various extracts and their potent biomolecules underlining the role of the plant proteins, an innovation, as therapeutic agents accredited with antiurolithiatic activity. The information in this review was obtained by searching PubMed, Web of Science, Scopus and Google Scholar for literature published, using terms like plant, urolithiasis, plant constituents and plant compounds. All abstracts and full text articles were thoroughly examined and most relevant articles were selected for inclusion in this review.

Renal stone disease is not a single disorder since stone composition varies which reflects constitutional,

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environmental and genetic factors. Table 1 lists the main types of renal stones and their relative prevalence[1]. Though the exact cascade of events leading to stone formation is unknown, it is postulated that urine is a supersaturated solution in which homogenous or heterogeneous nucleation leads to initial crystal formation, which can then aggregate and grow[5].

The pathogenesis of kidney stone formation is multifactorial and varies largely based on the stone phenotype. As other forms of mineralization, it encompasses several physico-chemical steps which occur either sequentially or concurrently.

Treatment and prevention of kidney stones has considerably evoluted during the last two decades by combination of dietary procedures, surgical treatments and medicaments, side effects of these methods and recurrence remain as problems to overcome. Thus, an adjunct to these conventional methods, phytotherapy is highly recommended.

Phytotherapy

Medicinal plants have been known for millennia and are being used as a rich source of therapeutic agents worldwide. WHO reported that ~75% global population, most in the developing world, depends on botanical medicines for their basic healthcare needs with around 800 plants being used in indigenous systems of medicines[8]. The use of herbal medicine is becoming popular due to toxicity and side effects of allopathic medicines.

Urolithiasis has been a matter of concern to clinicians since the time of Hippocrates. Many remedies have been employed during the ages to treat urinary stones. In the traditional system of medicine, most of the remedies were taken from plants and they proved to be useful though the rationale behind their use is not well established through systematic pharmacological and clinical studies except for some composite herbal drugs. The various marketed composite antiurolithiatic herbal formulations, Cystone (Himalaya Drug Company, India), Calcury (Charak Pharmaceuticals, Mumbai, India), Chandraprabhabati (Baidyanath, India), Neeri (Aimil Pharmaceuticals, India), Uriflow (BioNeutrix Healthcare, USA), Uriflush (Global Biosciences, Indonesia) and Culdisol (Ganga Pharmaceuticals, India) have been used worldwide.

Table 1—Main types of renal stones and their relative prevalence

Type of stones	Percentage prevalence
Calcareous stones (calcium oxalate and calcium phosphate stones)	75-90
Magnesium ammonium phosphate (struvite stones / infection stones)	10-15
Uric acid stones	3-10
Cystine and other stones	0.5-1

Scientific evidences

Concerning herbal medicines, there is a large number of plant species described in many pharmacopoeia worldwide as antiurolithiatic agents. Efforts are being made to elucidate the mechanism by which these plants exert their effect and identify active biomolecules involved. Table 2 lists the phytotherapeutic agents scientifically evaluated till date by various researchers.

Herniaria hirsuta, one of the most widely used herbal remedy, is a member of Caryophyllaceae, majorly found in Morocco. *H. hirsuta* progressively decreased the adhesion of calcium oxalate (CaOx) crystals to canine kidney cells by coating themselves at the crystal surface. In comparison to untreated rats, treatment with *Herniaria* decoction led to decreased urinary oxalate, fewer crystals in kidneys along with a decrease in size of crystals in urine. This suggests that the extract exhibits its potential by breaking the crystals and prevent retention of crystals as the excreted crystals were found to be CaOx dihydrate which bind less tightly to renal epithelia[9-11]. A bioactivity guided successive solvent extraction *in vitro* and *in vivo* was performed and it was observed that methanolic extract of aerial parts of *H. hirsuta* were quite effective both *in vitro*, using human urine and *in vivo* with male wistar rats. On further purification, this methanolic extract was found to be saponin rich revealing that saponins present in the plant are responsible for its antilithiatic potential[12]. *Boerhavia diffusa* and *Bryophyllum pinnatum* were effective by reducing the size and promoting the formation of CaOx dihydrate in place of CaOx monohydrate which are more injurious to renal epithelia[13].

Another important plant studied *in vitro* and *in vivo* for its antilithiatic potency is *Phyllanthus niruri*. It is used mainly in Brazilian folk medicine. Among various mechanisms of action postulated, *P. niruri* may modify crystal-crystal and/or crystal-matrix interaction[14], may also lead to higher incorporation of glycosaminoglycans (GAGs) in the calculi[15]. The beneficial effects of *Phyllanthus* in clinical studies done in 69 patients including males and females may

Table 2—Various phytotherapeutic agents used in the treatment of urolithiasis

Plant	Material	Model	Reference (no.)
Achyranthes aspera (Amaranthaceae)	Aqueous/ Roots	*In vitro*, cell lines	(31)
	Aqueous/ Roots	*In vivo*	(33)
Aerva lanata (Amaranthaceae)	Aqueous/ Leaves	*In vivo*	(41)
	Aqueous/ Aerial parts	*In vivo*	(42)
Alcea rosea (Malvaceae)	Hydro-alcoholic/ Root	*In vivo*	(70)
Ammi visnaga (Apiaceae)	Aqueous/ Seeds	*In vivo*	(37)
Musa paradisiaca (Musaceae)	Aqueous/ Stem	*In vivo*	(60)
Berberis vulgaris (Berberidaceae)	Aqueous Methanolic/ Root	*In vivo*	(58)
Berginia lingulata (Saxifragaceae)	Aqueous Methanolic/ Rhizomes	*In vitro*, *In vivo*	(17)
Boerhavia diffusa (Nyctaginaceae)	Ethanolic/ Plant	*In vitro*	(13)
Bombax cieba (Malvaceae)	Aqueous and Ethanolic/ Fruit	*In vivo*	(71)
Bryophyllum pinnatum (Crassulaceae)	Ethanolic/ Plant	*In vitro*	(13)
Commiphora wightii (Burseraceae)	Aqueous/ Root	*In vitro*	(34)
	Aqueous/ Herb	*In vitro*	(35)
Costus spiralis (Costaceae)	Aqueous/ Plant	*In vivo*	(62)
Vaccinium oxycoccos (Ericaceae)	Juice	Clinical trials	(49)
Crataeva nurvala (Capparaceae)	Aqueous/ Bark	*In vivo*	(27)
Cynodon dactylon (Poaceae)	Butanolic fraction and remnant, Ethyl acetate fraction/ Roots	*In vivo*	(67)
Citrus paradisa (Rutaceae)	Juice	*In vivo*	(50)
	Juice	Clinical trials	(51)
Camellia sinensis (Threaceae)	Aqueous/ Leaves	*In vivo*	(56)
Herniaria hirsuta (Caryophyllaceae)	Aqueous/ Herb	*In vitro*	(9)
	Aqueous/ Herb	*In vivo*	(10)
	Methanolic/ Aerial parts	*In vitro*, *In vivo*	(12)
Hibiscus sabdariffa (Malvaceae)	Aqueous/ Calyces	*In vivo*	(43)
Kampou extracts	Aqueous of 16 plants	*In vitro*, *In vivo*	(61)
Lagenaria siceraria (Cucurbitaceae)	Gum Acacia/ Fruit	*In vivo*	(73)
Leea macrophylla (Vitaceae)	Ethanolic/ Plant	*In vivo*	(74)
Citrus limon (Rutaceae)	Juice	*In vitro*	(47)
		In vivo	(48)
Moringa olifera (Moringaceae)	Aqueous and Alcoholic/ Roots	*In vivo*	(26)
Nigella sativa (Ranunculaceae)	Ethanolic/ Seeds	*In vivo*	(64)
Origanum vulgare (Lamiaceae)	Aqueous-methanolic/ Aerial parts	*In vitro*, Cell lines, *In vivo*	(36)
Orthosiphon grandiflorus (Lamiaceae)	Aqueous/ Leaves	*In vivo*	(53)
Paronychia argentea (Caryophyllaceae)	Aqueous and Butanolic/ Aerial parts	*In vivo*	(66)
Petroselinum sativum (Apiaceae)	Aqueous/ Aerial parts and Roots	*In vivo*	(75)
Phyllanthus niruri (Phyllanthaceae)	Aqueous/ Plant	*In vivo*	(14)
	Aqueous/ Plant	*In vivo*	(15)
	Aqueous/ Plant	Clinical trials	(16)
Pinus eldarica (Pinaceae)	Aqueous/ Fruit	*In vivo*	(68)
Plectranthus amboinicus (Lamiaceae)	Aqueous/ Leaves	*In vivo*	(63)

(*contd.*)

Table 2—Various phytotherapeutic agents used in the treatment of urolithiasis (*Contd.*)

Plant	Material	Model	Reference (no.)
Punica granatum (Lythraceae)	Juice/ Seeds	*In vivo*	(46)
	Methanolic and Chloroform/ Fruits	*In vivo*	(72)
Quercus salicina (Fagaceae)	Aqueous/ Leaves and Branches	Cell lines	(54)
	Aqueous/ Leaves and Twigs	*In vivo*	(55)
Raphanus sativus (Brassicaceae)	Aqueous/ Tubercles	*In vivo*	(40)
Rotula aquatica (Boraginaceae)	Aqueous/ Roots	*In vitro*	(34)
Rubia cordifolia (Rubiaceae)	Hydro-alcoholic/ Roots	*In vivo*	(57)
Salvadora persica (Salvadoraceae)	Aqueous and Alcoholic/ Leaves	*In vivo*	(69)
Sesbania grandiflora (Fabaceae)	Aqueous/ Twigs	*In vivo*	(59)
Solanum xanthocarpum (Solanaceae)	Hydro-alcoholic/ Fruits	*In vivo*	(44)
Terminalia arjuna (Combretaceae)	Butanolic/ Bark	*In vitro*	(25)
Terminalia chebula (Combretaceae)	Aqueous/ Fruit	*In vitro*, Cell lines	(32)
Tribulus terrestris (Zygophyllaceae)	Aqueous/ Fruit	*In vivo*	(19)
	Aqueous methanolic/ Fruit	*In vivo*	(20)
	Aqueous/ Fruit	*In vitro*, Cell lines	(21)
	Aqueous/ Fruit	*In vivo*	(22, 23)
	Butanolic/ Fruit	*In vitro*	(24)
Trigonella foenum-graceum (Fabaceae)	Aqueous/ Seeds	*In vivo*	(65)
Vediuppu chunam	Prepared from Vediuppu and Amai-odu	*In vivo*	(41)

be related to decreasing urinary crystallization promoters such as calcium[16]. The mechanism of the antiurolithiatic activity in *Berginia lingulata,* commonly called Prashanbheda, used in traditional Indian ayurveda system, mediated possibly through diuretic, hypermagnesuria and antioxidant effects[17]. A phenolic compound isolated from *Berginia cilita*, a member from same genus, effectively dissolved CaOx and calcium phosphate stones comparable to cystone *in vitro*[18].

Tribulus terrestris, commonly utilized herb in ayurveda for renal dysfunction, is proposed to be effective by decreasing urinary oxalate by alteration in oxalate synthesizing enzymes[19] and its 10% methanolic extract was found to be effective in preventing encrustation of implanted glass beads *in vivo*[20]. The aqueous extract of *T. terrestris* was cytoprotective towards NRK 52E (normal rat kidney epithelial cells)[21] with prophylactic and curative effect *in vivo*[22, 23] confirming the protective effect on the renal epithelial by the aqueous extract. n-butanol extract of *T. terrestris* was the most potent fraction *in vitro* similar to the effect of *Terminalia arjuna*, in bio-activity guided successive solvent extraction emphasizing the potential of saponins present in the plants[24,25]. Aqueous and ethanolic extracts of *Moringa olifera* Lam were found effective in decreasing the elevated levels of urinary oxalate *in vivo*, thus having a regulatory role in oxalate synthesis[26].

The use of *Crataeva nurvala* as a treatment for urolithiasis dates to 210 BC. The mechanism of action includes the reduction in glycolate oxidase, an oxalate synthesizing liver enzyme activity and renal oxalate crystallization with elevated urinary magnesium[27]. Lupeol (Lupa-21,20(29) dien, 3beta-ol), a pentacyclic triterpene isolated from *C. nurvala* showed prophylactic and curative activities in albino rats[28] and was found to be more effective than its structural analogue, Betulin (Lupa-20(29)ene-3,28 diol)[29] and the probable mechanism of action may involve the inhibition of CaOx crystal aggregation and enhancement of the body defence systems as they both have strong antioxidant potential[30]. *Achyranthes aspera* showed to be cytoprotective with no toxicity of its own[31], similar to *Terminalia chebula*[32] and was effective *in vivo*[33]. *Rotula aquatica* and *Commiphora wightii* in preliminary studies showed dissolution activity against urate stones[34], though *C. wightii* also prevented growth of Struvite crystals *in vitro*[35].

Khan *et al*.[36] explored the mechanism of antiurolithiatic potency of a traditional plant, *Origanum vulgare*, commonly called as oregano, which was found to be through inhibition of CaOx

crystallization, antioxidant, renal epithelial cell protective and antispasmodic activities. Antilithiatic potential of *Ammi visnaga*, a popular Saudi folk medicine, is attributed to its diuretic activity which results in decreasing supersaturation, thus reducing the risk of stone formation[37]. Phytoconstituents, khellin and visnagin isolated from *Ammi visnaga* fruits, further confirmed by HPLC were observed to be cytoprotective and effective against hyperoxaluric rats[38,39]. *Raphanus sativus*[40] was also found to be antilithiatic due to their diuretic potential. The efficacy of two siddha drugs, *Aerva lanata* and *Vediuppu chunam* as antilithic agents, were studied in rats. Along with reduction in oxalate synthesizing enzymes, urinary excretion of calcium, oxalate, uric acid, phosphorus and protein decreased significantly in treated group as compared to untreated rats with an increase in magnesium excretion which is a well known urinary inhibitor[41,42]. The aqueous extract of calyces of *Hibiscus sabdariffa* was found to be endowed with anti-urolithiatic activity without any genotoxic effects[43]. Saponin rich fraction from the fruits of *Solanum xanthocarpum* Schrad. & Wendl. (Solanaceae), a common folklore plant decreased the stone forming constituents in urine along with an increase in GAGs concentration[44].

Rosa canina, increased urinary citrate excretion, a remarked urinary stone inhibitor[45]. Juices being a major source of citrate have been explored by various groups for their antilithiatic potential *in vivo* using ethylene glycol for stone induction. Pomegranate *(Punica granatum)* juice was found to be effective by showing limited crystal growth in treated group along with increased iNOS and p65 expression emphasizing its antioxidant potential[46]. Lemon (*Citrus limon*)[47,48], cranberry (*Vaccinium oxycoccos*)[49] and grapefruit (*Citrus paradisa*) juices[50,51] deserve consideration as a conservative therapeutic protocol in managing calcium oxalate urolithiasis. Ingestion of cranberry juice altered urinary oxalate, phosphate and citrate levels in 20 South Africans showing its antilithogenic potential[49]. Urinary calcium, citrate and magnesium excretion was increased on administration of a soft drink containing grapefruit juice diluted (10%) in mineral water to 7 healthy subjects[51]. It is also proposed that citrus juices can be natural alternative for potassium citrate for being better tolerated and cost effective[50,51].

Orthosiphon stamineus has been used for the treatment of kidney and bladder stones and urinary tract infections attributed to diuretic, antiseptic and litholytic properties and its flavonoids were found to possess Adenosine A1 receptor binding activity, which induces diuresis and sodium excretion[52]. *O. grandiflorus* was also found to exhibit a protective potential in urolithiatic rat model due to its antioxidant properties[53]. Oxalate, has been shown to exert cytotoxic effects on renal tubular epithelial cells, attributable to increased oxidative stress within the cells. Antioxidant potential is proposed to be the mechanism of action of various antilithiatic plants like *Quercus salicina* Blume/ *Quercus stenophylla* Makino[54,55], *Camellia sinensis* (green tea)[56], *Rubia cordifolia* Linn[57], *Berberis vulgaris*[58], *Sesbania grandiflora*[59].

Different extracts of plants like banana (*Musa paradisiaca*) stem[60], Kampou extracts[61], *Costus spiralis*[62], *Plecranthus amboinicus*[63], *Nigella sativa*[64], *Trigonella foenum-graceum*[65], *Paronychia argentea*[66], *Cynodon dactylon*[67], *Pinus eldarica*[68], *Salvadora persica*[69], *Alcea rosea*[70], *Bombax cieba*[71], *Punica granatum*[72], *Lagenaria siceraria*[73], *Leea macrophylla*[74], *Petroselinum sativum*[75] have been evaluated for their antiurolithiatic potential *in vivo* and warrant further investigation to be established as preventive or therapeutic agents.

Rats treated with the hydro-alcoholic extract of *Copaifera langsdorffii* leaves showed increase in urinary magnesium with a decrease in urinary uric acid. There was a significant decrease in the calculi number and mass. HPLC profile of the extract identified flavonoids quercitrin and afzelin as the major components[76]. The antilithiatic and diuretic activity of 7-hydroxy-2′,4′,5′-trimethoxyisoflavone and 7-hydroxy-4′-methoxyisoflavone, isolated from aqueous extracts of the heartwood of *Eysenhardtia polystachya* collected from Mexico, was tested in rats and a decrease in calcium and magnesium in calculi composition was observed[77]. Berberine, an isoquinoline alkaloid, occurring in nature as the main constituent of several plants with medicinal use in kidney stone disease, was tested to understand the underlying mechanism. The compound with strong antioxidant potential, upon administration to hyperoxaluric rats showed increase in urinary pH along with sodium and potassium excretion and decrease in calcium excretion[78].

Until recently, pharmaceuticals used are being largely synthesized by organic chemistry. As knowledge about sources of many diseases and how

Table 3—Purified phytoconstituents with antiurolithiatic potential

Active Bio-constituents	Plant	Model	References (no).
Phenolic compound	*Berginia ciliata*	*In vitro*	(18)
Lupeol	*Crataeva nurvala*	*In vivo*	(28)
Betulin	*Crataeva nurvala*	*In vivo*	(29)
Khellin and Visnagin	*Ammi visnaga*	Cell lines, *In vivo*	(38, 39)
Quercitrin and Afzelin	*Copaifera langsdorffii*	*In vivo*	(76)
7-hydroxy-2′,4′,5′-trimethoxyisoflavone and 7-hydroxy-4′-methoxyisoflavone	*Eysenhardtia polystachya*	*In vivo*	(77)
Berberine	Constituent of several plants	*In vivo*	(78)
DAP (Protein)	*Dolichos biflorus*	*In vitro*	(85)
TAP (Protein)	*Trachyspermum ammi*	*In vitro*	(86)
		In vivo	(87)
TTP (Protein)	*Tribulus terrestris*	*In vitro*; cell lines	(88)

body fights these diseases is available, focus is on developing the therapeutics that mimic or enhance the actions of body's arsenal. Protein based drugs, as proteins are one of the main macronutrients in food, are one of the most important and rapidly growing segments of the pharmaceutical market with reduced immunogenicity, improved safety and greater effectiveness[79]. Insulin[80], plant lectins, Lunasin from soy[81], Bromelain from pineapple[82], MAP30 (*Momordica* anti-HIV protein of 30 kDa) and GAP31 (*Gelonium* anti-HIV protein of 31 kDa)[83], are few bioactive plant protein and peptides being explored.

Till date not many reports are available about antilithiatic plant proteins and peptides, even though urolithiasis has afflicted mankind since antiquity and there are many herbal formulations available in market. The antilithiatic plant proteins isolated, purified and characterized till date are anionic, rich in acidic amino acids and have EF Hand domain, a characteristic feature of various calcium binding protein like calgranulin, osteopontin[84]. Acidic amino acids interact with calcium ions thus making them unavailable for oxalate to bind. A 98 kDa dimeric antilithiatic protein was purified from seeds of *Dolichos biflorus* having abundant acidic amino acids. This protein showed similarity with calnexin of *Pisum sativum*[85]. A CaOx growth inhibitor with two EF hand domains was purified and characterized from seeds of *Trachyspermum ammi*[86]. The protein maintained renal functioning, reduced renal injury and decreased crystal excretion in urine and retention in renal tissues[87]. An antilithiatic protein (~14 kDa) isolated from *Terminalia arjuna* bark, a plant commonly found in Himachal Pradesh, showed promising results *in vitro*. A CaOx growth inhibitory protein isolated from *T. terrestris* (~60 kDa), anionic with EF hand domain, was found to be cytoprotective in comparison to cystone[88]. These proteins and peptides can be produced on large scale using recombinant DNA technology, taking into consideration potential toxicity, allerginicity and stability of peptides. Table 3 enlists the phytoconstituents with potential antiurolithiatic properties.

Conclusion

Medical management strategies include removal of stones and preventing recurrence. Based on the data obtained from *in vitro*, *in vivo* and clinical trials, it could be concluded that phytotherapeutic agents could serve as an alternative or adjunct therapy to available therapies. The reviewed studies reveal that few mechanisms of action of plants could be attributed to diuretic, increasing urinary citrate, antioxidant, antimicrobial, inhibitory properties of these plants. In the recent years, new proteins demonstrating biological activity towards urolithiasis, the third most common urinary disorder, have steadily been discovered. Future work includes development of a versatile expression system which ensures rapid and copious antilithiatic protein production along with maintenance of its activity and stability. Antilithiatic proteins from medicinal plants will open new vistas for using plant proteins as therapeutic agents to treat urolithiasis. *In vivo* and randomized controlled trails are also needed in order to evaluate the health potential of antilithiatic proteins.

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