Bio-active Compounds from Unani Medicinal Plants and Their Application in Urolithiasis

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M. K. Swamy, M. S. Akhtar (eds.), *Natural Bio-active Compounds*,
https://doi.org/10.1007/978-981-13-7205-6_16

Abstract

Botanical products are used in various forms, such as pure compound, standardized extract, etc., which are known for their remedial action against various diseases and also provide a lead for new drug development. Urolithiasis is a disease in which calcium oxalate has a major role. Pathogenesis of urolithiasis is multifaceted including numerous physicochemical events occurring concurrently and it includes supersaturation, nucleation, growth, aggregation and retention of crystals within the renal tubules. Composition of urine affects the incidence of urolithiasis resulting in alteration of biochemical parameters. The antiurolithiatic activity of several Unani plants has been studied. The antiurolithiatic phytoconstituents are phenolic compounds, saponins (solasodine), flavonoids (quercetin, kaempferol), alkaloids (crocin, berberine, khellin), tannins, other inorganic and organic constituents and plant proteins (glycosaminoglycans), etc. Studies have reported the role of ascorbic, citric, phytic, tartaric and oleanolic acid as good candidates for prophylactic management. Phytoconstituents exert their effects by multiple mechanisms. Oxalate causes lipid peroxidation through reacting with polyunsaturated fatty acids in cell membrane and damages the renal tissue. Triterpenes help in dissolution of oxalate crystals and demonstrate antioxidant effect. Organic substances adsorb on surface of the crystals and arrest the process of crystallization. Citrate and magnesium form a complex with calcium and decreases supersaturation; moreover, magnesium destabilizes CaOx pairs. Saponins help in disintegration of mucoproteins and reduce CaOx crystal adhesion to renal epithelial cells by pre-coating the crystals. Flavonoids significantly prevent the crystallization by antioxidant, anti-inflammatory and antimicrobial properties. The aim of writing this chapter is to highlight the bio-active compounds found in Unani medicinal plants in ameliorating the various stages of stone formation, and also to provide an overview of the use of plants in prevention and management of urolithiasis as well as elaborate its underlying mechanisms.

Keywords
Flavonoids · Medicinal plants · Natural compound · Unani medicine · Urolithiasis

16.1 Introduction

Since time immemorial, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants to treat various diseases (Savithramma et al. 2011). Herbal medicine includes active natural products mainly of low molecular weight and secondary metabolites. Plant secondary metabolites represent a treasure trove of therapeutic agents which are used by humans either for the treatment or management of numerous ailments of the body (Wink 2015). Natural products possess enormous structural and chemical diversity that continue to inspire novel discoveries in chemistry, biology and medicine. They are evolutionarily optimized as drug-like molecules and are the best sources of drugs and drug leads (Shen 2015). Traditional medicine offers a sea of opportunities for the development of various potential therapeutic agents which can be used either as extract or in combination with other herbs or as an isolated bio-active constituent (Saha and Verama 2013). Medicinal plants have already provided leads for potential antiparasitic, antifungal, antiviral and antibacterial compounds including flavonoids, coumarins, naphthoquinones, terpenoids, alkaloids, steroids, etc. (Sharma 2006).

Urolithiasis is referred to as stone formation in any part of the excretory system, viz. kidneys, bladder, ureters, and urethra, estimated to affect 12% population. The recurrence rate in female is 47–60% and in male 70–80% (Das et al. 2017). Epidemiological studies documented that urolithiasis is predominant in male (12%) than in women (6%) and is common among the ages of 20–40 in both male and female (Ghelani et al. 2016). Urolithiasis is the third most common disorder of the urinary tract with an estimated lifetime risk of around 1–5% in Asia, 8–15% in America and Europe and 20% in the Middle East countries (Panigrahi et al. 2016). 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 the USA (Aggarwal et al. 2014b). The prevalence of urolithiasis has been increased in India, and two high incidence stone belts had been identified (Panigrahi et al. 2016). The prevalence rate of urolithiasis in India is 15%. Recent studies have reported increased prevalence in the past decades within industrialized countries. This increasing trend is believed to be associated with changes in lifestyle modifications such as lack of physical activity, dietary habits and global warming (Alelign and Petros 2018; Ahmed et al. 2013a).

The treatments include extracorporeal shock wave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL) and drug treatment with considerable recurrence even after treatment. Although the surgical methods have improved to a great extent along with its cost, compelling data now suggest that exposure to shock waves in therapeutic doses may cause acute renal injury, decrease in renal function and an increase in stone recurrence. Additionally, persistent residual stone fragments and

possibility of infection after ESWL represent a serious problem in the treatment of stones. Also, even though drug treatment has shown some feasibility in many randomized trials, it has not been accomplished without side effects, which are sometimes very serious (Atmani et al. 2004). Herbal therapies are prevalent in various parts of the world for kidney calculi. Several plant origin remedies are employed through ages and are consumed in different dosage forms; some of them are experimentally (in vitro and in vivo) and clinically tested in search of putative alternative therapy. Unani system of medicine is widely practised in South Asia; all natural sources, i.e. plant, animal and mineral resources, are used as a drug to treat various diseases (Makbul et al. 2017). Unani medicine provides extensive details regarding *Hasate kulliya wa Masana* (urolithiasis) its pathogenesis, prevention and treatment. Many scholars such as Rhazes (850–925 CE), Ibn Sina (980–1037 CE) and Rabban Ṭabari (775–890 AD) have extensively discussed urolithiasis and its management. Rhazes has dedicated one whole chapter in *Kitab al-Hawi* (Continens Liber) on the pathology, signs, symptoms and drugs that can be used in the treatment of urolithiasis. The formation of stone anywhere in the body is due to viscous, adhesive, sticky matter. If this matter is exposed to heat, it get stuffed in the organs and is difficult to be excreted out from the body resulting in the accumulation and enlargement in size and finally transformed into a stone (Sina 2007; Majoosi 2010). Avicenna devoted a whole chapter in his book *The Canon of Medicine* and vividly discussed the principles for treating diseases of the excretory system and mainly for renal calculi (Faridi et al. 2014). According to Avicenna nephrolithiasis ally of phlegmatic matter, thick mucus, pus, and seldom blood stuff around particle, which are generally linked to the malfunction of kidney, obstruction, inflammation and excessive heat in the urinary tract (Faridi et al. 2012). Unani system of medicine manages calculi mostly by *Mufattite Hasat* (lithotriptic) and *Mudirre boul* (diuretic) drugs. Various single (Table 16.1) and polyherbal formulations like *Kushta Hajrul yahood, Majoon Hajrul yahood, Dawa-e-Sang, Majoon Sang Sarmahi, Safuf Hajrul yahood, Bunadiq-ul-Buzoor, Jawarish Zarooni, Majoon Aqra, Qurs kaknaj* and *Majoon Yadullah* (Makbul et al. 2017) are claimed to be antilithiatic and lithotriptic. The aim of penning this chapter is to emphasize the bio-active compounds found in Unani medicinal plants (Table 16.2) and their mechanism of action in ameliorating the various stages of stone formation and also to provide an overview of the underlying mechanisms of dietary plants as natural supplements in the management of urolithiasis.

16.2 Types of Urinary Stones

Based on the composition, kidney stones are broadly classified as calcium oxalate (70%), calcium phosphate (7–10%), uric acid (10%), struvite (15–20%) and cystine (1%) (Kumaran and Patki 2011). Many studies showed that calcium oxalate (CaC_2O_4) is the most important constituent of renal calculi. Two different types of CaC_2O_4 stones are formed: first is CaC_2O_4 monohydrate (COM) or whewellite and the other is CaC_2O_4 dihydrate (COD) or weddellite. COM is more frequently observed in clinical states as they are thermodynamically more stable and have greater affinity for renal tubular cells (Saha and Verma 2015b).

Table 16.1 List of plants and plant parts used in Unani system of medicine for the management of urolithiasis

Botanical name	Unani name	English name	Parts used
Acorus calamus	Waj	Sweet flag/calamus	Rhizome
Achyranthes aspera	Chirchita	Prickly chaff flower	Whole plant
Adiantum capillus-veneris	Parsioshan	Maiden-hair fern	Whole plant
Bergenia ligulata	Pakhanbed	Winter begonia	Rhizome
Bergenia ciliate	Pakhanbed	Winter begonia	Rhizome
Beta vulgaris	Chukandar	Beetroot	Bulb
Biophytum sensitivum	Chhuee Muee	Sensitive Plant	Whole plant
Bryophyllum pinnatum	Zakhme Hayat	Shrubby Basil	Leaves and seeds
Ocimum gratissimum	Franjmushk	Shrubby Basil	Leaves and seeds
Citrus medica	Lemun	Citron	Fruit
Citrus limon	Lemun	Lemon	Fruits
Centratherum anthelmenticum	Kalijiri	Purple fleabane, achenes	Seeds
Chenopodium album	Bathua	Bathua	Leaves
Dolichos biflorus, *Bergenia ligulata*	Kulthi and Pakhanbed	Horse gram	Seeds and rhizome
Jasminum auriculatum	Yasmeen flower	Jasmin flower	Flowers
Kalanchoe pinnata	Zakhme Hayat		Leaves
Lapis judaicus	Hajrul yahood	Jew's Stone	Stone
Moringa oleifera	Sahejna	Horseradish moringa	Legume, leaves, root bark
Melia azadirachta	Bakain	Bead Tree/Persian Lilac	Leaves, fruit, bark, flower and root
Musa paradisiaca	Chhuee Muee	Sensitive plant	Whole plant
Nerium oleander	Kaner	Kaner pant	Flower, root and leaves
Nigella sativa	Kalonji	Black seed	Seed
Nymphaea alba	Nilofar	Water Lily	Flower, seeds and root
Olives europaea	Zaitoon	Olive	Fruit and leaves
Peucedanum grande	Duqu	Peucedan fruit	Fruits
Piper longum	Filfil-e-daraz	Long pepper	Fruit
Plantago major	Bartang	Broadleaf plantain/ plantain tree	Seeds
Punica granatum	Rumman	Pomegranate	Fruits, leaves and bark
Phoenix dactylifera	Chhuara	Dry dates	Fruit
Phyllanthus niruri	Bhui Amla	Bhui Amla	Whole plant
Raphanus sativus	Mooli	Radish	Whole plant
Rotula aquatica	Pakhanbed	Winter begonia	Rhizome
Rubia cordifolia	Majeeth	Indian Madder	Rhizome
Solanum xanthocarpum	Katai Khurd	Wild eggplant/yellow berried nightshade	Acrid

(continued)

Table 16.1 (continued)

Botanical name	Unani name	English name	Parts used
Terminalia chebula	Halela	Chebulic Myrobalan	Fruits
Terminalia arjuna	Arjun	Arjuna tree	Bark
Tinospora cordifolia	Gilo	Gulancha tinospora	Whole plant
Tribulus terrestris	Khar-e-Khasak	Caltrops	Fruit
Trianthema portulacastrum	Biskhapra	Hogweed	Whole plant
Gymnema sylvestre	Gudmar buti	Cowplant/Australian	Leaves
Trigonella foenum-graecum	Methi	Trigonella seed	Seeds
Ammi majus	Atrilal	Bishop's weed	Seeds
Withania somnifera	Asgand	Winter cherry root	Root
Zeya mays	Maize	Indian maize	Corn silk

16.3 Aetiological Factors of Urolithiasis

The factors responsible for the formation of stone in the urinary tract include hot climate, excessive use of protein-rich meat, prolonged immobilization, decrease in urinary citrate, inadequate urinary drainage and genetic derangements (Ahmed et al. 2013a; Kumar et al. 2016). Supersaturation of urine occurs in geographically high temperate regions, an important factor for urolithiasis which finally increases urine crystallization. Various other factors such as pH, ionic strength, certain glycoproteins and urinary solute concentration are also liable for supersaturation (Monti et al. 2016; Patel et al. 2010). Frequent recurrence is the characteristic of urolithiasis particularly in untreated metabolic disorders, recurrent UTI, anatomical defects or inadequate hydration (Monti et al. 2016).

Inadequate water consumption is also one of the major causes of kidney stone. Acidification of urine occurs in a person who consumes excessive meat protein which leads to increased elimination of oxalates, calcium and uric acid (Ahmed et al. 2013b). The most common metabolic abnormality observed in patients of calcium stone is hypercalciuria though it may be due to other diseases such as primary hyperparathyroidism. Hyperoxaluria, hyperuricosuria, hyperphosphaturia and hypocitraturia are other metabolic disorders which increase risk of kidney stone formation (Zerwekh 2002). As far as aetiopathogenesis of urolithiasis is concerned, there are a lot of similarities between Unani and conventional medicine. The causes mentioned in conventional medicine has vividly discussed by Unani scholars thousands of years ago. Most of the renowned physicians were of common opinion to state that excessive heat and morbid viscous humours are the key factors for the formation of stone. Further they mention that concentrated urine is among the most important factor of urinary calculi which is closely correlated to the modern concept of supersaturation of urine. Additionally, the consumption of heavy diet rich in thick milk, paneer (fresh cheese) and fried meat produces thick viscous matter in the body especially when digestive power is weak. Galen (129–200 CE) mentioned that ulcer in the kidney is one of the aetiologies for urinary calculi. Randall's plaque is the extended version of Galen's theory (Makbul et al. 2017).

Table 16.2 Major chemical constituents present in antiurolithiatic Unani medicinal plants

Plant names	Saponin	Flavonoids	Tannin	Alkaloid	Phenolic compounds	References
Acorus calamus	+	+	+	+	–	Imam et al. (2013)
Achyranthes aspera	+	–	–	+	–	Srivastav et al. (2011)
Adiantum capillus-veneris	–	+	–	–	–	Ahmed et al. (2012)
Bergenia ligulata	–	+	+	+	+	Gurav and Gurav (2014)
Bergenia ciliate	–	+	–	–	+	Singh et al. (2017)
Beta vulgaris	+	+			+	Miraj (2016)
Biophytum sensitivum	+				+	Pawar and Vyavahare (2014)
Bryophyllum pinnatum and *Ocimum gratissimum*	+	+	+	+	+	Afzal et al. (2012) and Agarwal and Varma (2014)
Citrus medica	–	+	–	+	–	Panara et al. (2013)
Centratherum anthelmenticum	+	–	–	–	–	Amir and Chin (2011)
Chenopodium album	+	+	+	+	+	Kumar and Kumar (2015)
Citrus limon	–	+	–	–	+	Mohanapriya et al. (2013)
Dolichos biflorus	+	+	+	–	–	Alok et al. (2014)
Kalanchoe pinnata	+	+	+	+	–	Rajsekhar et al. (2016)
Moringa oleifera		+		+	–	Anwar et al. (2007)
Musa paradisiaca		+	+		–	Imam and Akter (2011)
Nerium oleander	+	+	+	+	–	Kiran and Prasad (2014)
Nigella sativa	+	+		+	–	Ahmad et al. (2013)
Nymphaea alba	–	–	+	+	–	Prajapati et al. (2009)
Olea europaea	–	+	–	–	–	Hashmi et al. (2015)
Peucedanum grande	+	+	–	–	+	Kumar, et al. (2016)
Piper longum		+	+	+	+	Patel et al. (2011)

(continued)

Table 16.2 (continued)

Plant names	Saponin	Flavonoids	Tannin	Alkaloid	Phenolic compounds	References
Plantago major	+			+		Samuelsen (2000)
Punica granatum	–	+	–	–	–	Rahimi et al. (2012) and Prasad and Kunnaiah (2014)
Phoenix dactylifera	–	+	+	–	–	Ateeq et al. (2013)
Phyllanthus niruri	–	+	+	–	–	Kamaruzzaman and Haq (2016)
Raphanus sativus	+	+	–	+	–	Ushakiran (2017)
Rotula aquatica	–	+	+	+	+	Vysakh et al. (2016)
Rubia cordifolia	–		+	–	+	Verma et al. (2016)
Solanum xanthocarpum	+	+		+		Reddy and Reddy (2014)
Terminalia chebula	–	–	+	+	+	Rathinamoorthy and Thilagavathi (2014)
Terminalia arjuna	+	+	+	+	+	Jain et al. (2009)
Tinospora cordifolia		–	–	+	–	Saha and Ghosh (2012)
Tribulus terrestris	+	+	–	+	–	Chhatre et al. (2014)
Trianthema portulacastrum	+	+	+	+	+	Sree et al. (2014);
Gymnema sylvestre	+	+	+	+	+	Thakur et al. (2012)
Trigonella foenum-graecum	+	+	–	+	+	Goyal et al. (2016) and Bano et al. (2016)
Ammi majus	+	+	–	+	+	Al-Asnafi (2013) and Selim and Ouf (2012)
Withania somnifera	+	+	–	+	–	Mishra et al. (2000) and Saiyed et al. (2016)
Zea mays	+	+	+	+	–	Milind and Isha (2013)

16.4 Process of Stone Formation

Urolithiasis is a multistep progressive disorder which occurs in a sequential manner with several physicochemical events, i.e. supersaturation of urine, formation of solid crystal form and growth, aggregation and retention of crystals within the renal tubules (Ahmed et al. 2016). Multiple theories are proposed by researchers for kidney stone formation such as crystallization precipitation theory which states that supersaturation of urine leads to precipitation of stone crystallites. These critical particles are entrapped, and subsequent crystal growth follows (Dharmaraj et al. 2006). According to the inhibitory theory normal urine is composed of inhibitory substances that prevent the stone formation by inhibiting the crystallization and growth of calcium oxalate crystals (Dharmaraj et al. 2006), while free particle theory states that under supersaturation crystal, nuclei are formed in the lumen of the nephron by homogenous nucleation. Subsequently these nuclei would enlarge and eventually retained in the lumen of the distal nephron leading to obstruction. The fixed particle theory also requires crystal nuclei formation in the lumen of the nephron and adherence to apical surface of the tubular epithelium (Dharmraj et al. 2006). Recent studies have shown that calcium oxalate kidney stones form as overgrowth on apatite plaques in the renal papillae called Randall's plaques. It provides an excellent surface for heterogeneous nucleation. Randall's plaques began in the deep medulla in the basement membrane of the thin line loop of Henle and then spread through the interstitium to the basement membrane of the papillary urothelium. If the urothelium becomes damaged, the plaque is exposed to the urine, and calcium oxalate crystals form on the plaque, accumulating a clinically significant mass to form a stone (Longo et al. 2012; Pfau and Knauf 2016). According to vascular theory that is concerned with the development of Randall's plaque has hypothesized the coincidence of kidney stones with diabetes, hypertension or arteriosclerosis. Diabetic patients often show oxalate in their urine; further low urine pH increases the risk for uric acid calculi (Pfau and Knauf 2016).

16.5 Stages of Urolithiasis

16.5.1 Nucleation

The formation of a solid crystal mass is called as nucleation; if it is formed in a pure solution, it is known as homogenous nucleation. Secondary nucleation results in the mass production of crystals, where new crystals deposit on similar type of pre-existing crystal surfaces. Epitaxy is clinically important in the formation of calcium oxalate stones. Urine is a mixture of various solutes and solvents; therefore heterogeneous nucleation in urine frequently occurs over an existing surface or an alternative structure. Epithelial cells, red blood cells, cell debris, urinary casts, other crystals and bacteria in urine can be heterogeneous nucleation sites (Basavaraj et al. 2007).

16.5.2 Crystal Growth

The driving force for crystallization is due to reduction in the potential energy of the atoms or molecules when they form bonds to each other. The crystal growth process starts with the nucleation stage. Several atoms or molecules in a supersaturated liquid start forming clusters; the bulk free energy of the cluster is less than that of the liquid. Crystal growth is determined by the size, shape and characteristics of the material, supersaturation levels, pH and structural defects in the crystals. Growth of the crystal is imperative for particle formation (Basavaraj et al. 2007).

16.5.3 Crystal Aggregation (Crystal Agglomeration)

Crystal aggregation is more important factor in all the steps of stone formation than nucleation and growth, because aggregation occurs within seconds. Crystals stick together in a solution and forms a larger particle. Crystal aggregation is determined by a balance of forces, with aggregating and disaggregating effects. It depends upon the interparticle distance; small interparticle distance enhances the attractive force and favours aggregation. Additionally, Tamm-Horsfall glycoprotein and other molecules may act as bonding agent and increase attraction among them. Furthermore, aggregate may be stabilized by solid bridges formed by crystalline material connecting two particles. The main force that inhibits aggregation is the repulsive electrostatic surface charge, known as Zeta potential (Basavaraj et al. 2007).

16.5.4 Crystal Retention

For urolithiasis it is essential that crystals should be accumulated and retained in the kidney. Retention takes place due to the association of crystals with the epithelial cells lining in the renal tubules. Crystal retention depends on the composition of epithelial cell surface of renal tubules. Normally the epithelial surface of the urinary system is non-adherent and serves as a natural defence against adhesion of any particle. Nevertheless, if it is damaged due to any injury or infection, its non-adherent property is lost which acts as a nidus for foreign particles (Basavaraj et al. 2007).

16.6 Role of Promoters and Inhibitors in the Pathogenesis of Stone Formation

Kidney stones are a complex of crystals and organic materials often known as matrix. Matrix is composed of urinary macromolecules which play an important role in the formation of kidney stones (Khan and Kok 2004). Inhibitors are molecules which increase supersaturation necessary to start nucleation, but they reduce the rate of crystal growth and aggregation and inhibit secondary nucleation as well. On the contrary, a promoter reduces the formed product of the supersaturated

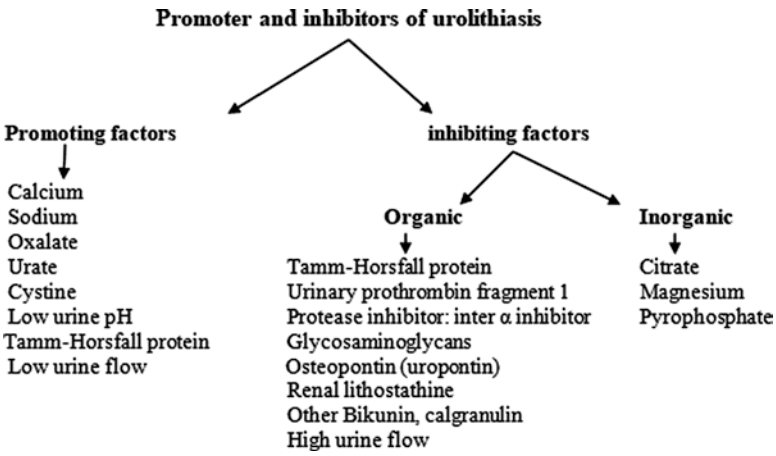

Fig. 16.1 Promoters and inhibitors of urolithiasis

solution. An imbalance between promoters and inhibitors are more significant than a disturbance of any single substance (Fig. 16.1) for the formation of urinary stone (Moe 2006; Basavaraj et al. 2007).

16.6.1 Citrate

Citric acid circulates in blood in the form of tricarboxylic acid, which at pH of 7.4 make a complex to calcium, magnesium and sodium. Majority of the circulating citrate which is derived from endogenous oxidative metabolism are freely filtered by glomerulus. From which 75% of the filtered citrate is reabsorbed in the proximal convoluted tubule. Crystallization of calcium oxalate monohydrate and calcium phosphate is perhaps altered by citrate. Citrate acts both through surface controlled mechanisms to hamper crystal growth and aggregation and also by the formation of stable soluble complexes with calcium (Basavaraj et al. 2007; Jawalekar et al. 2010). Citrate inhibits COM crystal growth at above 0.1 mM concentrations that is range of its concentration in the loop of Henle. Hypocitraturia is found in a majority of stone formers. Alkali therapy is found useful in hypocitraturic patients by increasing the ability to inhibit crystal aggregation and also by increasing urinary pH. This in itself might be useful as citrate and pyrophosphate are more effective at pH around 7 (Khan and Kok 2004).

16.6.2 Pyrophosphates

Normal pyrophosphate level in urine is 20–40 μM; this high level is adequate to inhibit CaOx and CaP crystallization. However, it was observed that pyrophosphate at concentration of 16 μM inhibits COM crystal growth. Pyrophosphate and

diphosphate are known to stall the precipitation of CaP; in addition diphosphates have also an ability to inhibit growth of apatite crystals (Basavaraj et al. 2007). Pyrophosphate and bisphosphonates act on crystal aggregation but in a complex manner. Pyrophosphate inhibits crystal aggregation in dose-dependent manner. Bisphosphonates act by a combined action of the two phosphonate groups and side chains in close proximity, which bind to the crystal surface. The side groups have increasing affinity for calcium which increased the capacity to inhibit crystal growth (Khan and Kok 2004).

16.6.3 Magnesium

Dietary magnesium (Mg) is absorbed by small intestine which is excreted through kidneys, except 1% which takes part in the composition of blood. Magnesium can form complexes with oxalate and decreases supersaturation. Similar to calcium, oral ingestion of Mg decreases the oxalate absorption and urinary excretion by means of binding to oxalate in the gut. Magnesium supplementation increases citrate excretion in urine (Basavaraj et al. 2007).

16.6.4 Osteopontin (Uropontin)

Osteopontin (OPN) is an aspartic acid-rich protein; it regulates the mineralization both physiologically and pathologically and inhibits the crystal growth. OPN is a phosphorylated protein of wide tissue distribution that is found in association with dystrophic calcification including in the organic matrix of kidney stones. It is synthesized in the kidney and presents in urine involved in various biological processes (inflammation, leukocyte recruitment, wound healing and cell survival). Preclinical study suggested that OPN may hinder calcium oxalate crystallization and growth as well as inhibit their adhesion to cultured epithelial cells (Basavaraj et al. 2007; Khan and Kok 2004).

16.6.5 Urinary Prothrombin Fragment 1

The blood clotting factor prothrombin is degraded into three fragments: thrombin, fragment 1 and fragment 2. Fragment 1 is excreted in urine and is named urinary prothrombin fragment (UPTF1) and is a potent inhibitor of CaOx stone formation in vitro. UPTF1 is an important inhibitor of CaOx crystal aggregation and crystal adherence to renal cells. As reported in some previous studies, sialylated glycoforms of UPTF1 shield the body against CaOx stone formation probably by coating the surface of crystals (Khan and Kok 2004).

16.6.6 Tamm-Horsfall Protein

Tamm-Horsfall protein (THP) is the most plentiful protein in the urine of normal mammals. It is a type of glycoprotein which is synthesized exclusively in the thick ascending limb of the loop of Henle with exception of the macula densa. THP takes part in the pathogenesis of cast, urolithiasis, nephropathy and tubule interstitial nephritis. It was observed that after the administration of high-protein diet to rats, THP was significantly increased in urine. However, controversy exists regarding its effect on crystal aggregation; most authors argue that THP inhibits COM crystal aggregation in a solution which has high pH, low ionic strength and low concentration of divalent ions. On the other hand, low pH and high concentrations of calcium, sodium and hydrogen ions lost its inhibitory effect, and it may even turn into a promoter (Basavaraj et al. 2007; Khan and Kok 2004).

16.6.7 Glycosaminoglycans

Glycosaminoglycans (GAGs) is one of the macromolecules present in the stone matrix. The most prominant GAGs are heparan sulphate and hyaluronic acid excreted in urine which are supposed to play a vital role in CaOx crystallization (Khan and Kok 2004; Basavaraj et al. 2007).

16.6.8 Renal Lithostathine

Lithostathine is immunologically related to pancreatic protein that inhibits the growth of calcium carbonate crystals which may promote heterogeneous nucleation. It is normally present in healthy person urine and in renal stones (Basavaraj et al. 2007).

16.6.9 Bikunin

This was isolated from urine of rats and bovine kidneys and identified as urinary bikunin, the light chain of inter-α-inhibitor (IαI). Its presence in organic matrix of crystals and kidney stones indicates that it may fulfil a directive role in lithogenesis. This protein inhibits CaOx crystallization efficiently (Atmani 2001).

16.6.10 Phytate

Calcium oxalate crystallization could be prevented by the excessive consumption of whole cereals and legumes as they have sufficient amount of phytate. It was observed that stone formers excrete low amount of phytate in comparison to healthy individuals (Grases et al. 2006).

16.6.11 Promoters

Various inorganic compounds, proteins, and glycosaminoglycans are known as stone promoters. Promoters and inhibitors are in equilibrium in non-stone formers, but if kidney function is disturbed or concentration of urinary constituents is altered due to any reason, it changes the physicochemical state of urine and ultimately disturbs the set equilibrium of promoters and inhibitors which leads to stone formation. The presence of cell debris, protein aggregates and other crystals on the cell surfaces of the kidney may offer equivalent site for nucleation. These nucleation sites possibly will lower the supersaturation essential to kick off crystallization, as a result promoting CaOx crystallization. Evidence suggests that uric acid and CaP may promote heterogeneous nucleation. Calcium may promote formation and growth of intrarenal crystals. Hypercalciuria can decrease inhibitor action that leads to crystallization. Moreover newly produced crystals and factors that modulate crystal cell interactions may possibly motivate the initiation of an intrarenal stone (Basavaraj et al. 2007).

16.7 Treatment of Urolithiasis

Urolithiasis needs both preventive and curative therapy. Various treatment options are available for every type of stone. Moreover, evidence suggests that recurrence rate of urolithiasis can be reduced by treating specific biochemical abnormalities (Bijauliya et al. 2017; Ghelani et al. 2016).

16.7.1 Thiazide Diuretics

It decreases reabsorption of sodium by inhibiting the NaCl co-transporter in the distal convoluted tubules and increased calcium reabsorption. The hypocalciuric effect of thiazide diuretics can be enhanced by restrictive consumption of sodium which also minimizes potassium losses caused by the use of thiazides (Gul and Monga 2014).

16.7.2 Potassium Citrate

Potassium citrate decreases the risk of recurrent calcium stone formation in patients with low urinary citrate. This therapy leads to a significant increase in urinary citrate, pH and potassium which ultimately lowers the risk of stone formation (Bijauliya et al. 2017).

16.7.3 Allopurinol

Allopurinol prevents the production of uric acid by acting on enzyme xanthine oxidase as competitive inhibitor that converts xanthine into uric acid. Though, the effect of allopurinol treatment in patients without hyperuricosuria has not been established, hyperuricosuria is not a necessary prerequisite for allopurinol therapy (Bijauliya et al. 2017).

16.7.4 Cholestyramine

It is used in case of enteric hyperoxaluria to reduce oxalate hyperabsorption. It reduces the irritating effect of free bile acids on the colonic mucosa. Moreover, it has been shown to bind oxalate in in vitro (Pfau and Knauf 2016).

16.7.5 Sodium Cellulose Phosphate

It reduces the intestinal absorption of calcium as a result of which normal calcium excretion is restored. It also has a capability to induce hypermagnesemia by reducing the complexation of urinary oxalate and magnesium, thereby leading to increase saturation of CaOx (Pfau and Knauf 2016).

16.7.6 Penicillamine (Cuprimine)

It is often prescribed in the treatment of cystine stone of those patients who do not show any response even after drinking of more fluids (Pfau and Knauf 2016).

16.7.7 Bisphosphonates

It efficiently decreases fasting calciuria and minimally decreases 24 h calciuria (Pfau and Knauf 2016).

16.7.8 Potassium Phosphate

It produces its effect by increasing serum phosphate and urine phosphate and also increases urinary pyrophosphate. The alternative to manage urolithiasis is surgery that includes PCNL and ESWL, but these techniques do not prevent recurrence. Furthermore, they are not risk free; acute renal injury, hypertension, haemorrhage, tubular necrosis and subsequent renal fibrosis are frequent aftereffects (Ghelani et al. 2016).

16.8 Prevention of Urolithiasis

Effective kidney stone prevention depends upon addressing the reason of formation. Generally, to prevent first episodes for kidney calculi formation or its secondary episodes, proper management of diet and the use of medications are required. Primary prevention of kidney stone disease via dietary intervention is low-cost public health initiative with massive societal implications. Thus, nutritional management is the best preventive strategy against urolithiasis (Alelign and Petros 2018). The risk of stone formation can be effectively reduced by increasing fluid intake (at least 4 l/day) and more than 2 or 2.5 l/day urine output. The deficit fluid intake leads to low urine output and supersaturation of urine with various solutes (Grases et al. 2006; Gul and Monga 2014; Han et al. 2015; Pfau and Knauf 2016). Uses of fruit juices prevent stone formation by increasing urine volume; moreover fruit juices are rich in potassium and citric acid. Citrate binds with urinary calcium thereby reduced the supersaturation of urine. In addition, it binds calcium oxalate crystals and prevents crystal growth. Lemon juice (4 oz) per day significantly increases urine citrate level without increasing oxalate level. Melon and orange juice are also rich sources of citrate (Saxena and Sharma 2010; Gul and Monga 2014). Vitamin C in large dosage increases urine oxalate concentration since it is metabolized to oxalate. Therefore, its excessive use should be restricted. In contrast vitamin B6 (pyridoxine) may reduce urinary oxalate. Pyridoxine is a cofactor for alanine-glyoxylate aminotransferase (AGT) enzyme that catalyses the conversion of glyoxylate to glycine. It was observed that a deficiency of AGTor low levels of pyrodoxine lead to conversion of glyoxalate in oxalate. It was concluded through previous studies that an inverse relationship exist between vitamin B6 intake and the risk of stone formation (Gul and Monga 2014). The active ingredient of fish oil is eicosapentaenoic acid (EPA) which is an n-3 fatty acid. Prostaglandin E2 (PG_{E2}) decreases by increasing EPA n-6 fatty acid metabolites. Decreased hyperoxaluria and increased renal calcium reabsorption take place by lower levels of PG_{E2} due to the activation of the nephron Na/K/2Ca transporter (Gul and Monga 2014).

16.9 Management of Urolithiasis by the Major Compounds of Unani Medicinal Plants

Plants secondary metabolites have been used for centuries in traditional medicine due to their large biological activities. Phytotherapy utilizes extract which contains large number of secondary metabolites often from several structural groups. In most cases, it was nearly impossible to define single phytoconstituent, which could explain the bioactivity of the extract. Pharmacological activity of an extract may be due to synergistic interactions of various compounds that cannot be detected when evaluated alone (Wink 2015). Several studies demonstrated that flavonoids, triterpenoids and saponins, viz. a-amyrin, b-amyrin and lupeol from different plants, showed antiurolithiatic and diuretic activity (Dinnimath et al. 2017). The antiurolithiatic activity of several Unani medicinal plants has been studied. The

phytoconstituents responsible for antiurolithiatic activity are phenolic compounds, saponins, flavonoids, alkaloids, tannins, other inorganic and organic constituents, plant proteins, etc. (Kasote et al. 2017; Fig. 16.2).

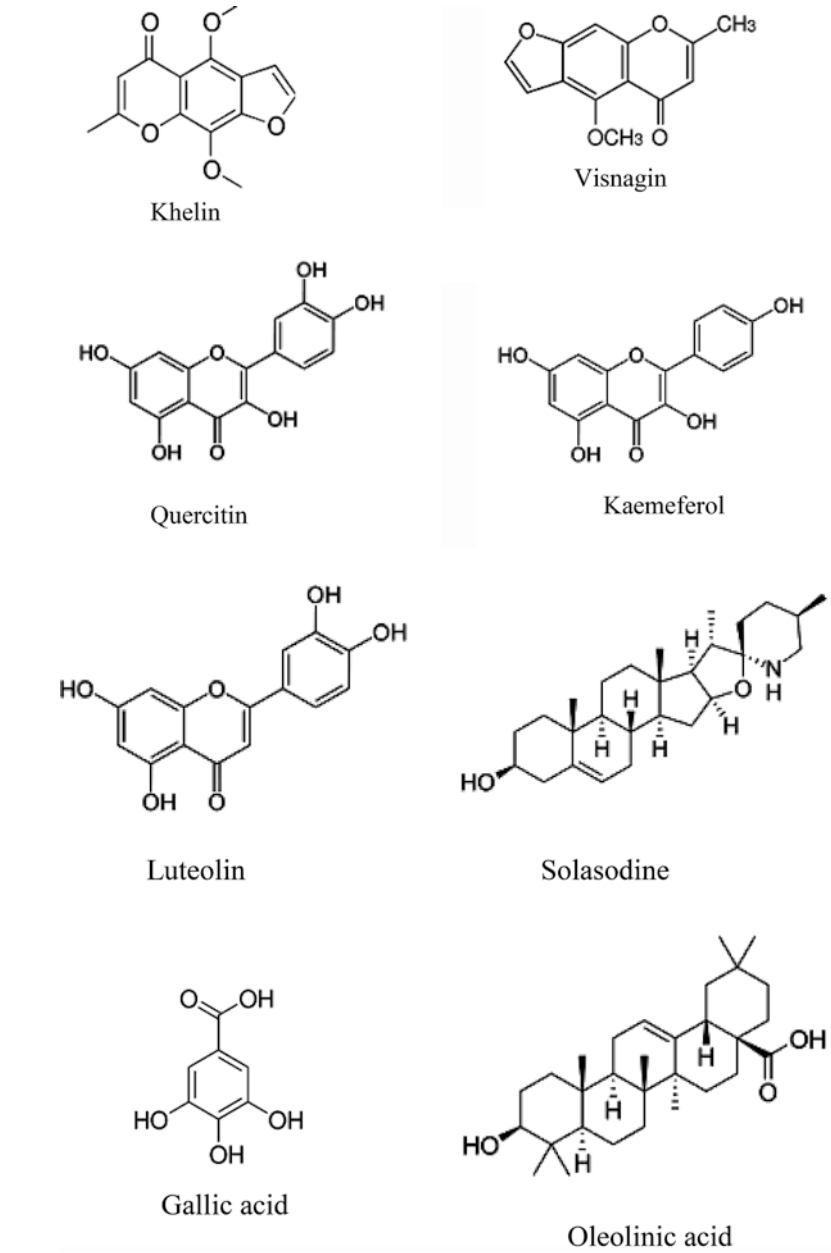

Fig. 16.2 Chemical structure of some phytoconstituents

16.9.1 Alkaloids

Alkaloids are a class of nitrogen-containing organic products present in plants, fungi and bacteria. A number of alkaloids isolated from medicinal plants experimentally demonstrate anti-proliferation, antibacterial, analgesic, antiviral, insecticidal and anti-metastatic effect (Shi et al. 2014; Saxena et al. 2013). *Ammi visnaga* L. extract significantly reduces the occurrence of CaOx crystal deposition and increases citrate excretion in urine followed by hypo-oxaluria (Vanachayangkul et al. 2011). Further Khella extract (KE) increases the urine pH in dose-dependent manner, beside an increase of urine volume. It may be assumed that KE inhibits the reabsorption of citrate due to change in pH; as a result it could lead to the prevention of CaOx crystal formation in the kidney. The detailed mechanism of action of *Ammi visnaga* seeds was not yet elucidated. However, it may be due to bio-active constituent khellin and visnagin that can prevent renal tissue injury caused by CaOx crystals. Further, more than one mechanism has been proposed by various researchers, viz. the action of *Ammi visnaga* to be attributed to its vasodilating and diuretic properties or the effect may be due to khellin which interferes with the citrate metabolism in inhibiting the CaOx crystallization (Bhagavathula et al. 2015). In addition to this khellin promotes smooth muscle relaxation, diuresis and affects urinary citrate which may have pleiotropic effects on urolithiasis. Ghaeni et al. (2014) reported that Crocin isolated from *Crocus sativus* L. showed significant reduction in urine protein excretion and less or no crystals in animals of prophylactic treatment. Crocin demonstrate its effect at initial phases of calculi formation (Ghaeni et al. 2014).

Berberine is an alkaloid found in many medicinal plants and used in the treatment of urolithiasis. Traditional systems of medicine frequently utilize berberine-based formulations for the treatment of diverse pathological states of the body. It has demonstrated various pharmacological actions such as antimicrobial, antihypertensive, anti-inflammatory, antioxidant, antidepressant, anticancer, antidiarrhoeal, cholagogic, hepatoprotective and nephroprotective (Singh et al. 2010). The compound administration to hyperoxaluric rats showed increase in urinary pH along with sodium and potassium excretion and decrease in calcium excretion (Aggarwal et al. 2014). The fraction of various extracts of *Nigella sativa* L. showed preventive effect on ethylene glycol-induced nephrolithiasis. The chief non-polar compound of *Nigella sativa* L. seeds extract is thymoquinone which has strong preventive and extremely disruptive effect on CaOx crystallization with decreased excretion of oxalate in urine. Thymoquinone not only inhibits inflammatory products but also inhibits cyclooxygenase and 5-lipoxygenase pathways. Furthermore, it also has antioxidant and antimicrobial effect (Hadjzadeh et al. 2007).

16.9.2 Flavonoids

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom (Agrawal 2011). Quercetin, kaempferol, morin,

myricetin and rutin are acknowledged with antioxidant, antidiabetic, anticancer, anti-inflammatory, antimicrobial, antiviral, hepatoprotective, cardioprotective and neuroprotective activities (Sharma 2006; Hsu et al. 2017; Wang et al. 2018). Flavonoids also possess enzyme inhibition, oestrogenic, anti-allergic, vascular and cytotoxic and antitumor activities (Saxena et al. 2013). Studies documented that flavonoids obtained from diverse plant sources are effective in urolithiasis (Soundrarajan et al. 2006; Butterweck and Khan 2009). The flavonoids by virtue of their antioxidant, anti-inflammatory and antimicrobial activities prevent the stone formation and dissolute the already formed crystals (Kumar et al. 2016). Aqueous extract of *Bergenia ligulata* contains quercetin (4.2%); nevertheless more than 1.5% quercetin-containing extract may possibly exert a potent antiurolithiatic agent (Sharma et al. 2017). In vitro antiurolithiatic effect of *Bergenia ciliata* was evaluated by Byahatti et al. (2010), and he found that isolated crude phenolic compound has maximum dissolution in contrast to alcoholic extract, butanol and ethyl acetate fractions of both calcium oxalate and phosphate calculi (Byahatti et al. 2010). Quercetin; kaemepferol triterpenes, viz. betulin; and tannins help in dissolution of calcium oxalate crystals as well as display antioxidant effect (Sikarwar et al. 2017). Antioxidant potential is the best-described property of almost each group of flavonoids. The sequential order of scavenging activity of flavonoids is myricetin> quercetin > rhamnetin > morin > diosmetin > naringenin > apigenin > catechin >5, 7-dihydroxy-3′,4′,5′-trimethoxyflavone > robinin > kaempferol> flavones (Narayana et al. 2001; Tapas et al. 2008). Luteolin and catechins are better antioxidants than the nutrient antioxidants, viz. vitamin C and E and β-carotene. Quercetin prevents cyclooxygenase and lipoxygenase actions consequently declining the formation of arachidonic acid which is inflammatory metabolites (Agrawal 2011). *Dolichos biflorus* L., a well-known lithotriptic drug, has registered varied flavonoids such as quercetin, b-sitosterol, streptogenin, a phytohaemagglutinin, b-N-acetylglucosaminidase, a- and b-galactosidases, a-mannosides and b-glucosides (Saha and Verma 2015a, b). It is also reported that the administration of rutin and curcumin restores to normal levels of the elevated calcium and oxalate in the urine and kidney. Histopathological study showed less tissue damage and fewer calcium oxalate deposition in the kidney. Numerous studies evaluated the anti-inflammatory and antioxidant potential of rutin and curcumins, so its role in urolithiasis may be part of it (Ghodasara et al. 2010).

Aqueous extract of *Bauhinia variegate* showed inhibition of growth of crystals in dose-dependent manner which may be because of highest amount of flavonoids (54.6 mg/g equivalents of quercetin). However ethanolic extract of *Bauhinia variegate* showed maximum amount of tannins (56.30 mg/g equivalents to quercetin) and equal amounts of alkaloids (25 mg/g equivalents of atropine sulphate). Whereas steroids and saponins are present in lowest amount (Mamillapalli et al. 2016). Quercetin and betulin of *Aerva lanata* increased excretion of urine volume, significantly reduced the size of calculi and reduced calcium, oxalate and phosphate excretion; moreover, an increase in magnesium level was also reported. Further remarkable reduction in BUN and creatinine level in test group animals was also observed (Dinnimath et al. 2017). Quercetin and betulin were also evaluated by docking with

a protein 2 ETE of *Oxalate oxidase*, and the results indicated better regiospecificity with the enzyme (Dinnimath and Jalalpure 2015). Diosmin is a type of citrus bioflavonoid, mostly found in citrus fruits often used as a dietary supplement. It exhibits anti-inflammatory and antioxidant activities. The administration of diosmin to urolithiatic rats efficiently recovered the elevated serum parameters, kidney weight, urine pH and urine calcium and phosphorus to normal levels (Prabhu et al. 2016). Another study has shown that diosmin reduced deposition of calcium oxalate and tissue degeneration in rats (Noorafashan et al. 2013; Saha and Verma 2015b).

A number of flavonoids displayed antibacterial activity, viz. complete growth inhibition of *Staphylococcus aureus* by quercetin. Citrus flavonoid (hesperidin) showed significant anti-inflammatory and analgesic effect. Recently apigenin and luteolin are reported to have anti-inflammatory activity. LO and COX inhibitory activities were showed by kaempferol, quercetin, myricetin and fisetin (Tapas et al. 2008). Study demonstrated that methoxy flavonoids of *Orthosiphon grandiflorus* can induce diuresis and excretion of sodium by blocking the adenosine A1 receptor. Since this receptor is found in the glomerulus, proximal tubules, collecting ducts and afferent arterioles, its antagonist acts either directly by inhibiting sodium reabsorption in the proximal tubules or indirectly by dilatation of afferent arterioles (Vanachayangkul et al. 2011).

16.9.3 Tannins

Tannin-containing plant extracts are used as astringent, diuretic and exert anti-inflammatory, antiseptic, antioxidant and haemostatic properties (Saxena et al. 2013). Aqueous extract of *Bergenia ligulata* showed quercetin (4.2%) in maximum and then tannic acid, gallic acid, and catechin. Tannic acid was found to be abundant marker in best bio-active dichloromethane (DCM) fraction. Catechin may protect the calculi formation by preventing oxalate-induced oxidative injury (Sharma et al. 2017). *Bergenia ligulata* either increases bioavailability of nitric oxide (which activates cGMP that controls intracellular calcium level) or activates enzymes like lactate dehydrogenase and glycolic acid oxidase that catalyse redox reaction of glyoxylate into glycolate and oxalate (Sharma et al. 2017).

16.9.4 Saponins

Saponins are group of secondary metabolites, non-volatile surfactants that are commonly found in plant, in lower marine animals and in some bacteria. Saponins possess haemolytic, pesticidal, molluscicidal, antimicrobial, insecticidal, anthelmintic, analgesic, anti-inflammatory, sedative, antitumor, antidiabetic, antifungal, antiviral, antiparasitic and immunomodulatory activities (Hassan et al. 2012). Saponins are also widely used in the pharmaceutical industry as adjuvant to enhance absorption

of other drugs by increasing solubility or interfering in the mechanisms of absorption (Barbosa 2014). Saponin-rich fractions of *Herniaria hirsuta* L. inhibited calcium oxalate crystallization both in vitro and in vivo (Sikarwar et al. 2017). The study of Patel et al. (2012) documented that *Solanum xanthocarpum* fruit which is saponin-rich demonstrates diuretic and stone-dissolving action. In vivo result also showed calcium oxalate crystal inhibition in different stages of stone formation. Solasodine content in saponin-rich fraction was found to be 0.658% by HPTLC. Similar results were obtained in *Bergenia ligulata*, *Trachyspermum ammi*, *Tribulus terrestris*, *Achyranthes aspera* and *Beta vulgaris* (Saranya and Geetha 2014). Studies indicated that mucoproteins possess considerable affinity toward CaOx crystal surface, hence promoting their growth (Patel et al. 2012). Saponin disintegrates the mucoproteins, promoters of crystallization, and also decreases adhesion of CaOx crystal to renal epithelium by means of pre-coating (Saha and Verama 2013, 2015b; Sikarwar et al. 2017).

16.9.5 Plant Proteins

Organic matrix consisting of different proteins is also found in many plants, which are supposed to play a significant role in growth and modification of crystal form. A study reported the presence of four proteins in the organic matrix of calcium oxalate found in the seeds of *Phaseolus vulgaris*; these isolated proteins do not only inhibit the nucleation of CaOx crystals but also modify their morphology (Bijarnia et al. 2009). Some of the antilithiatic plant proteins isolated, purified and characterized till date are anionic, are rich in acidic amino acids and have EF-hand domain like calgranulin and osteopontin distinguishing trait of various calcium-binding proteins. Acidic amino acids interact with calcium ions making them unavailable for oxalate to bind. Protein from *Dolichos biflorus* showed crystallization inhibition activity against calcium oxalate and calcium phosphate. On the contrary some of the studies argue that nonprotein part of *Dolichos biflorus* is responsible for antilithiatic activity. However, it has been demonstrated that Asp and Glu which are the residues of acidic amino acid change their nature in acidic urine and turned into negatively charged ion which are attracted to positively charged COM ions. Moreover, chemical analysis of amino acid also proved that a protein DAP contains higher concentration of acidic amino acids such as Asp and Glu in a similar fashion as found in CNX. Hence it is concluded that DAP which is similar to CNX protein has potential to inhibit calcium oxalate crystallization. The protein maintains kidney functions, reduces tissue damage and decreases excretion and retention of crystals in kidneys as well. An antilithiatic protein (~14 kDa) isolated from *Terminalia arjuna* bark showed promising results in vitro. A CaOx growth inhibitory protein isolated from *Tribulus terrestris* L. (~60 kDa), anionic with EF hand domain, was found to be cytoprotective (Aggarwal et al. 2014).

16.10 Possible Pharmacological Actions Responsible for Antiurolithiatic Activity

Urine is a complex mixture of crystalloids (oxalate, uric acid, calcium and cystine) and colloids (mucin and sulphuric acid) which are present in equilibrium and in dissolved state. Any disturbances in this equilibrium such as increase in crystalloid or decrease in colloid contents or vice versa lead to formation of renal stone. Unani lithotriptic drugs are boasts of several bio-active constituents which act through diverse mechanisms on the different stages of stone formation. Some of the drugs mentioned in the literature are being practised by traditional healers and have demonstrated a number of significant effects like diuretic, antioxidant, anti-inflammatory, analgesic, antimicrobial, nephroprotective, etc. Studies on animal models further revealed the fascinating multidimensional action of plants accountable to its effect in urolithiasis.

The aetiopathogenesis of urolithiasis includes oxidative stress, injury, inflammation, etc. The two signalling molecules reactive oxygen species (ROS) and reactive nitrogen species (RNS) play a very important role in stone formation. The main source of ROS in the kidney is NADPH oxidase. It was experimentally observed that when renal surface comes into contact of high amount of oxalate, calcium oxalate and calcium phosphate crystals, it becomes more active and shows increased gene expression and production of those molecules which are responsible for tissue alteration, inflammation as well as biomineralization. In urolithiasis due to increased excretion of oxalate in urine and its deposition in the kidney, renin-angiotensin system is upregulated and generates angiotensin II, as a result of which NADPH oxidase is activated which leads to the production of reactive oxygen species or reactive nitrogen species. ROS injuries cause cell death and form membrane-bound vesicles which help in crystal nucleation (Panigrahi et al. 2016). When peroxidation increases and thiol content decreases oxalate-binding process ultimately increases; consequentially nucleation and aggregation of stone matrix aggravate. This activity is also linked with peroxidized mitochondria and nuclei suggesting that the peroxidation can be a contributing factor for the initiation of stone formation (Ramezani et al. 2009; Ahmed et al. 2013c).

Phenolic compounds and flavonoids are the natural antioxidants with antimutagenic and anti-inflammatory properties. However, the antioxidant potential of lemon juice is not only due to the presence of flavonoids (eriocitrin, hesperetin and limonoids) but many other constituents such as citrate, vitamin C and vitamin E. Vitamin E may prevent hyperoxaluria-induced peroxidative damage to renal tubular membrane and calcium oxalate crystal deposition and subsequent development of kidney stones (Touhami et al. 2007). Level of lipid peroxides was restricted with treatment of isolated lupeol and botulin from *Crataeva nurvala* Buch Ham. This might be attributed to the ability to reduce the level of oxalate supersaturation by diuretic activity. Moreover, it may be cytoprotective by providing protection against free radical-induced derangements (Anand et al. 1994; Dinnimath and Jalalpure 2015). Many antioxidants are found in Unani medicinal plants (Table 16.3) containing some of the ingredients which have oxidizable functional groups.

Table 16.3 Pharmacological activities of antiurolithiatic Unani drugs

Plant name	Anti-inflammatory	Antioxidant	Antimicrobial	Analgesic	Diuretic	Nephroprotective	Spasmolytic	References
Acorus calamus	+	+	+	+	–	–	+	Imam et al. (2013)
Achyranthes aspera	+	+	+	+	+	+	–	Srivastav et al. (2011)
Adiantum capillus-veneris	+	+	+	+	–	–	–	Ahmed et al. (2012, 2013a)
Bergenia ciliate	+	+	+	+	–	–	–	Singh et al. (2017)
Beta vulgaris	+	+	+	–	–	–	–	Miraj (2016)
Biophytum sensitivum	+	+	+	+	–	–	–	Pawar and Vyavahare (2014)
Bryophyllum pinnatum	+	+	+	+	–	+	–	Afzal et al. (2012)
Citrus medica	–	–	+	+	–	–	–	Panara et al. (2013)
Centratherum anthelmenticum	+	–	+	+	+	–	–	Amir and Chin (2011)
Chenopodium album	+	+	+	+	–	–	+	Sikarwar et al. (2013); Kumar and Kumar (2015)
Citrus limon	–	+	+	–	–	–	–	Mohanapriya et al. (2013)
Dolichos biflorus	–	+	–	–	–	–	–	Alok et al. (2014)
Kalanchoe pinnata	+	+	+	+	+	+	+	Rajsekhar et al. (2016)
Moringa oleifera	+	+	+	+	+	–	+	Anwar et al. (2007)
Melia azadirachta	+	+	+	+	–	–	–	Hwisa et al. (2014)
Musa paradisiaca	–	+	+	–	+	–	–	Imam and Akter (2011)
Nerium oleander	+	–	+	–	+	–	–	Kiran and Prasad (2014)
Nigella sativa	+	+	+	+	+	+	+	Ahmad et al. (2013)

(continued)

Table 16.3 (continued)

Plant name	Anti-inflammatory	Antioxidant	Antimicrobial	Analgesic	Diuretic	Nephroprotective	Spasmolytic	References
Olea europaea	+	+	+	+	–	–	–	Hashmi et al. (2015)
Peucedanum grande	–	–	–	–	+	–	–	Kumar et al. (2016)
Plantago major	+	+	–	+	+	–	–	Samuelsen (2000)
Punica granatum	+	+	–	–	–	–	–	Rahimi et al. (2012)
Phoenix dactylifera	+	+	+	–	–	+	–	Ateeq et al. (2013)
Phyllanthus niruri	+	+	+	+	+	–	+	Kamaruzzaman and Haq (2016)
Rotula aquatica	+	+	+	+	–	–	–	Vysakh et al. (2016)
Rubia cordifolia	+	+	+	+	–	+	–	Verma et al. (2016)
Solanum xanthocarpum		–	–		–	–	–	Patel et al. (2012)
Terminalia chebula	+	+	+	+	+	+	+	Pawar et al. (2012)
Terminalia arjuna	+	+	+	–	–	–	–	Chaudhary et al. (2010)
Tinospora cordifolia	+	+	+	+	+	+	+	Kumar et al. (2011)
Tribulus terrestris	+	–	+	+	+	–	+	Chhatre et al. (2014)
Trianthema portulacastrum	+	+	+	+	+	–	+	Sree et al. (2014)
Gymnema sylvestre	+	+	+	+	+	–	+	Thakur et al. (2012)
Trigonella foenum- graecum	–	+	+	–	–	–	+	Al-Asnafi (2013)
Ammi majus	–	+	+	–	–	–	+	Selim and Ouf (2012)
Zea mays	+	+	–	–	+	+	–	Milind and Isha (2013)

Antioxidant polyphenols act as nephroprotective by interfering with the generation of free radicals (Lien et al. 2012).

Soundararajan et al. (2006) reported that ethylene glycol is liable to increase deposition of calcium, oxalate and phosphate in kidneys. The increase in calcium level may due to the increased bioavailability of nitric oxide (NO) in succession activate cGMP (30, 50-cyclic guanosine monophosphate) that controls intracellular calcium levels. As reported in previous studies, NO donors have the capability to control it, and perhaps this is one of the reasons behind the preventive effect of anti-urolithiatic drugs. Furthermore ethylene glycol treatment increased oxalate production by activation of oxalate-synthesizing enzymes which catalyse the oxidation and reduction of glyoxylate into glycolate and oxalate (Soundararajan et al. 2006). These changes smooth the progress of hyperoxaluria and subsequent crystal adherence and retention in renal tubules (Byer and Khan 2005). Thus herbal drug either by inhibiting oxalate synthesis or by increasing the bioavailability of NO can effectively control the levels of both salts.

Antioxidant potential is proposed to be the mechanism of action of various antilithiatic plants (Bahuguna et al. 2009; Pawar et al. 2012; Aggarwal et al. 2014). *Punica granatum* L. contains phenols that are potent antioxidants with three times more effective than red wine or green tea (Ebadi 2009). The antioxidant effects of flavonoids in green tea and *Orthosiphon grandioxorum*, *Acorus calamus*, *Peucedanum grande*, *Solanum xanthocarpum*, *Tribulus terrestris*, *Punica granatum*, etc. (Table 16.3) decreased oxidative injury and deposition of calcium oxalate in kidneys of rats. Tannins, flavonoids and isoflavonoids exert their effect through antioxidant and may lead to relaxation of smooth muscle of the excretory and biliary tract. This might help for expulsion and reduction in size of calculi in rats (Saha and Verma 2015a, b; Ghelani et al. 2016).

Diuretic activity is an essential feature in urolithiasis treatment. Since an increase in volume of fluid pass age through the kidney will help in dissolving and passing of stone thus evading further retention and flushing out the deposits. Quercetin and betulin were potent antiurolithiatic found to be associated with the diuretic activity (Jagannath et al. 2012; Dinnimath et al. 2017). The effect may be produced by stimulating regional blood flow or initial vasodilatation or by producing inhibition of tubular reabsorption of water and anions, with the result in both cases being dieresis (Hailu and Engidawork 2014). Antiurolithiatic Unani drugs (Table 16.3), viz. *Achyranthes aspera* L., *Bergenia ligulata* (Wall.) Engl, *Centratherumanthelmenticum* L., Kuntze, *Kalanchoe pinnata* Pers., *Moringa oleifera* Lam., *Peucedanumgrande* C.B. Clarke, *Phyllanthus niruri* L., *Terminalia chebula* Retz., *Tinospora cordifolia* (Wild.) Miers, *Tribulus terrestris* L., *Trianthema portulacastrum* L. and *Zea mays* are reported to possess diuretic activity. Ethanolic pulp extract of *Citrullus lanatus* showed antiurolithiatic and diuretic actions. GC–MS analysis confirmed the presence of steroids and alkanes (Siddiqui et al. 2018). Steroids reduce the calculus-induced distal ureter inflammation and submucosal oedema and are considered as important components of medical expulsive therapy. As reported in some previous studies, A1 receptor antagonists can induce dieresis and sodium excretion (Orhana et al. 2015). *Orthosiphon stamineus* has been used in urolithiasis and UTI

attributing to its diuretic, antiseptic and litholytic properties. Its flavonoids were also found to possess adenosine A1 receptor-binding activity, which induces diuresis and sodium excretion (Aggarwal et al. 2014).

Though each step of the stone formation is crucial, growth of stone is most stressing in clinical practice. When stone increases in size, it obstructs renal passage resulting in colicky pain. At this situation analgesic, antispasmodic and anti-inflammatory drugs are used for symptomatic relief. Obstruction does not only disturb urine out flow but glomerular filtration rate is also decreased which further lead to formation of nitrogenous substances (Ahmed et al. 2013c; Ghelani et al. 2016). Several experimental studies showed that kidney function is normalized with administration of various plant extracts mentioned in Table 16.4. Additionally, it was observed in a study carried by gel growth method that in bacterial infection, urine pH increases, and a "biofilm" forms which favours the formation of organic component of stone. It is also anticipated that high pH and metal-binding ability of the biofilm are independently responsible for supersaturation (Das et al. 2017). Unani medicinal plants (Table 16.3) have antimicrobial action against various bacterial strains. Therefore, antimicrobial activity could be considered as one of the possible mechanisms for test drug to evolve an antilithogenic agent. Moreover, a number of medicinal plants contain glycosaminoglycans (GAGs) which are inhibitors of calcium oxalate crystallization. Stones are formed when there is imbalance in the equilibrium of inhibitors and promoters. When these conditions favour the calculi formation, antiadherent layer of GAGs acts as a protective barrier. Damage of this layer due to outcome of bacterial attack in the nucleus will develop leading to a full-fledged stone in the excretory tract. At this point of time, the drugs having antimicrobial property may be effective which protect antiadherent layer by covering the epithelium of collecting system.

Diet strongly affects the urinary pH. Rich in animal protein diet is related to high excretion of uric acid in urine and a low urine pH. Solubility of uric acid decreases considerably at urine pH below 5.5, which leads to formation of crystal that is able to act as a heterogeneous nucleant for calcium oxalate crystals. Citrate-rich products and carbonated beverages markedly increase urinary pH. Solubility of calcium phosphate abruptly decreases at above pH value 6.0, responsible for the formation of calcium phosphate crystals that can act as heterogeneous nucleant for calcium oxalate crystals (Grases et al. 2006). pH of urine is raised by citrate and can also decrease calcium excretion in urine and bind calcium in a soluble complex, resulting in reduction in calcium salt supersaturation. In addition, citrate inhibits crystal formation, growth and aggregation (Atmani 2003; Monti et al. 2016). Berberine, an isoquinoline alkaloid, has strong antioxidant potential. Upon administration to hyperoxaluric rats, an increase in urinary pH along with sodium and potassium excretion and decrease in calcium excretion were noted (Aggarwal et al. 2014). On the other hand, the excretion of citrate in urine has been reported to be elevated after administration of magnesium and that its basic pH could be another inhibitor of stone formation (Basavaraj et al. 2007; Ghaeni et al. 2014).

Table 16.4 In vitro, in vivo and clinical study on Unani medicinal plants for its antiurolithiatic activity

Plant name	Extracts used	Type of study	Method of urolithiasis induction	References
Acorus calamus	Ethanolic	Male Wistar rats	Ethylene glycol (0.75% v/v)	Ghelani et al. (2016)
Achyranthes aspera	Ethanolic	Male Wistar rats	0.75% of ethylene glycol	Awari et al. (2009)
Adiantum capillus-veneris	Hydroalcoholic	In vitro male S.D. rats	EG + AC (0.75% + 1%), respectively	Ahmed et al. (2013a)
Bergenia ligulata	Ethanolic and aqueous methanolic	In vitro, ex vivo female Wistar rats	Ethylene glycol (0.75% v/v)	Sharma et al. (2017)
Bergenia ciliate	Hydroalcoholic	In vitro	CaOx crystallization by calcium chloride and sodium oxalate solution	Saha and Verma (2013)
Bergenia ciliata	Alcohol, ethanol, ethyl acetate fraction	In vitro	Calcium oxalate and calcium phosphate stones by homogenous precipitation and semipermeable membrane from farm eggs	Byahatti et al. (2010)
Beta vulgaris	Aqueous	In vitro	Nucleation, aggregation and growth assay	Saranya and Geetha (2014)
Biophytum sensitivum	Methanolic	male Wistar rats	EG + AC (0.75% + 1%), zinc disc implantation (20 ± 2 g)	Pawar and Vyawahare (2015)
Bryophyllum pinnatum and *Ocimum gratissimum*	Aqueous	In vitro	Nucleation assay in synthetic urine	Pinjarkar et al. (2017)
Citrus limon	Lemon juice	Male Wistar rats	Ethylene glycol and ammonium chloride (0.75% v/v and 2% w/v)	Touhami et al. (2007)
Centratherum anthelmenticum	Methanolic	In vitro	CaOx crystallization by calcium chloride and sodium oxalate solution	Galani and Pancha (2014)

(continued)

Table 16.4 (continued)

Plant name	Extracts used	Type of study	Method of urolithiasis induction	References
Chenopodium album	Methanolic and aqueous	In vitro, Wistar rats (both sexes)	CaOx crystallization by calcium chloride and sodium oxalate solution. EG 0.75%	Sikarwar et al. (2017) and Sharma et al. (2016a)
Dolichos biflorus	Hydro methanolic	In vitro	CaOx crystallization by calcium chloride and sodium oxalate solution	Saha and Verma (2015b) and Atodariya et al. (2013);
Dolichos biflorus and *Bergenia ligulata*	Aqueous, detannated and deproteinized aqueous	In vitro	By homogenous precipitation method	Garimella et al. (2001)
Jasminum auriculatum	Aqueous and alcoholic	Male albino rats	(0.75% v/v)	Bahuguna et al. (2009)
Kalanchoe pinnata	Aqueous	In vitro	CaOx and calcium phosphate stones by homogenous precipitation and semipermeable membrane from farm eggs	Phatak and Hendre (2015)
Lapis judaicus	Powder	Clinical trial	DRCS on 60 patients	Faridi et al. (2014)
Citrus limon and *Citrus sinensis*	Juice	In vitro	Calcium oxalate monohydrate crystallization by calcium chloride, sodium oxalate, sodium chloride and sodium acetate	Kulaksizoglu et al. (2008)
Moringa oleifera	Aqueous and alcoholic	Male Wistar rats	Ethylene glycol (0.75%)	Karadi et al. (2006); Fahad et al. (2010)
Melia azadirachta	Aqueous	male Wistar rats	Zinc disc (4 mm diameter weighing around 40–50 mg)	Hwisa et al. (2014)
Musa paradisiaca	Aqueous- ethanol	Male Wistar rats	EG + AC (0.75% + 1%), respectively	Panigrahi et al. (2017)
Nerium oleander	Ethanol	Male Wistar rats	Ethylene glycol (0.75%)	Suman et al. (2017)
Nigella sativa	Ethanolic	Male Wistar rats	Ethylene glycol (1% v/v)	Hadjzadeh et al. (2007)

(continued)

Table 16.4 (continued)

Plant name	Extracts used	Type of study	Method of urolithiasis induction	References
Nymphaea alba	Ethanolic	Male Wistar rats	Zinc disc	Bhaskar and Shelke (2012)
Olea europaea	Olive oil	Male mice	Ethylene glycol (0.75% v/v)	Alenzi et al. (2017)
Peucedanum grande	Hydroalcoholic	Male S.D. rats	EG + AC (0.75% + 1%), respectively	Kumar et al. (2016)
Piper longum	Alcoholic and aqueous	In vitro	Nucleation and aggregation assay	Patel et al. (2011)
Plantago major	ethanol	In vitro	Modified Schneider slide gel method	Aziz et al. (2005)
Punica granatum	Chloroform and methanolic	Male Wistar rats	ethylene glycol (0.75% v/v)	Rathod et al. (2012)
Phoenix dactylifera	Hydro alcoholic	Male Wistar rats	Ethylene glycol (0.75% v/v)	Al-Gamali et al. (2017)
Phyllanthus niruri	Petroleum ether, ethyl acetate, methanol and water	In vitro	Calcium oxalate crystal dissolution and turbidity method	Khare et al. (2014)
Raphanus sativus	Aqueous	In vitro, male Wistar rats	Ethylene glycol (0.75% v/v)	Akhtar et al. (2017)
Rotula aquatica	Aqueous	In vitro, male Wistar rats	CaOx crystallization by calcium chloride and sodium oxalate solution Ethylene glycol (28 days)	Sasikala et al. (2013) and Vijayakumari et al. (2017)
Rubia cordifolia	Hydroalcoholic	Rats	EG + AC (0.75% + 1%), respectively	Divakar et al. (2010)
Solanum xanthocarpum	Ethanol-water	Male Wistar rats	Ethylene glycol (0.75% v/v)	Patel et al. (2012)
Terminalia chebula	Aqueous	Male Wistar rats	EG + AC (0.75% + 1%)	Pawar et al. (2012)
Terminalia arjuna	Aqueous	In vitro	Calcium phosphate crystallization by $KH_2O_4 + CaCl_2$ and calcium oxalate crystallization by calcium chloride and sodium oxalate	Chaudhary et al. (2010)

(continued)

Table 16.4 (continued)

Plant name	Extracts used	Type of study	Method of urolithiasis induction	References
Tinospora cordifolia	Ethanol	In vitro	Crystallization assay in synthetic urine	Kumar et al. (2011)
Tribulus terrestris	Aqueous	In vitro	Nucleation and the growth of the calcium oxalate (CaOx) crystals	Sharma et al. (2016b) and Aggarwal et al. (2010)
Trianthema portulacastrum and *Gymnema sylvestre*	Ethanolic	Male Wistar rats	Ethylene glycol and ammonium chloride (0.75% and 1%, respectively)	Sree et al. (2014)
Trigonella foenum- graecum, and *Ammi majus*	Watery suspension of powder	Sprague Dawley rats	Ethylene glycol (3%) in diet	Ahsan et al. (1989)
Withania somnifera	Methanolic	Male Wistar rats	EG + AC (0.75% + 1%), respectively	Patel and Mandal (2014)
Zeya mays	Aqueous	In vitro	Calcium oxalate crystallization by calcium chloride anhydrous and sodium oxalate	Rathod et al. (2013)

Urine is a by-product of metabolism having ions that are constantly interacting with calcium and phosphate. Its presence in urine acts as inhibitor for calcium salt crystallization (Jawalekar et al. 2010). Calcium complexes with citrate hence reduce crystallization of calcium salts in vitro. In another study it was found that citrate has promoted crystal nucleation although reduced growth. Hennequin et al. (1993) using a different model of crystallization confirm this fact. Previous in vitro studies reported that it fix at the surface of the crystals to reduce their size and modify their shape. Another study mentioned that citrate selectively sets on some crystalline faces and had a strong activity against crystal aggregation (Oussama et al. 2005). In another report, citrate increases t_{max} and reduces rates of nucleation and aggregation in a non-concentrating manner. This is opposite to of Hess et al. (2000) result. In vitro study on lemon and orange juices tested on the calcium oxalate crystallization significant inhibiting effect of lemon juice was observed. Golde et al. documented lemon juice is richest in citrate. Citrate is six times more concentrated in the lemon juice than the orange juice, as an inhibitor impact of lemon juice is higher than that of orange juice. This is in agreement with the observations that orange juice increased t_{max}, but it did not alter the rate of nucleation and aggregation significantly (Kulaksizoglu et al. 2008).

One of the key steps in the processes of nephrolithiasis is the transformation of retained crystals to concrete stones in the lumen of renal tubules. The interactions among CaOx crystal and cells hinder by Mg and OPN. Mg may possibly act as an inhibitor, while OPN may inhibit COM nucleation, growth reduction and

aggregation (Zhong et al. 2012). It was observed that rats with high magnesium containing diets were protected from the deposition of crystals in the kidney. Magnesium forms complex with oxalate to form soluble complex and powerfully inhibits the crystallization in vitro. Magnesium also inhibits absorption and excretion of oxalate thus prevents its supersaturation and consequently reduces the growth and nucleation rate (Soundrarajan et al. 2006; Saranya and Geetha 2014; Dinnimath and Jalalpure 2015; Pawar and Vyawahare 2017). Quercetin and betulin reduce the risk of calcium oxalate urolithiasis by a significant excretion of magnesium in urine (Dinnimath and Jalalpure 2015). On the other hand, potassium ammonium citrate efficiently prevents the recurrence of stone in patients (Saranya and Geetha 2014; Pawar and Vyawahare 2017).

Hammarsten's classic study states that several ions like Mg^{2+}, citrate, etc. are generally present in urine which increase solubility of CaOx in aqueous solutions. However clinical study does not show promising results, as they are metabolized in the organism. In a study, it is reported that amino acids in a minimum concentration in urine significantly inhibit the growth of CaOx crystals. One more study reported that a component of urine, a-ketoglutaric acid, has the ability to inhibit the crystal growth and increase the solubility of calcium oxalate crystals in different physiological solutions; a sizable amount of a-ketoglutaric acid is present in urine which forms a weak and comparatively unstable chelate complex with calcium. But the process of dissolution of CaOx with a-ketoglutaric acid is quite slow and takes around 1 month to dissolve stone. However, its potential as an effective clinical solvent of calcium oxalate crystals should be assessed according to the situation, e.g. excessive water intake continuously flushes the calculus with urine; at this situation dissolution effect will be shorter. In a study, the effect of a-ketoglutaric acid and amino acid on growth inhibition was observed in which a-ketoglutaric acid was found to be more effective. The better effect of a-ketoglutaric acid may be due to its capability to lower the supersaturation (Atanassova et al. 1996).

Oleanolic acid reduces supersaturation of the urine by increasing dieresis. This action of oleanolic acid could be due to the activation of muscarinic receptor in the bladder muscles along with other mechanisms (Vyas and Argal 2013). Oleanolic acid and ursolic acid showed potent antihypertensive, diuretic and natriuretic activity (Freitas et al. 2011). The different mechanisms proposed by different workers are either prevention or dissolution or reduction in size of stones in their subtle constituents (Fig. 16.3). This further demonstrates that the Unani medicinal plants produced effect through diverse mechanism complementing each other.

16.11 Conclusions and Future Prospects

Antiurolithiatic activity has been documented to numerous phytochemicals from Unani medicinal plants. Different categories of phytochemicals flavonoids, tannins, saponins, alkaloids, steroids, plant acids and plant proteins have been identified. Pharmacological studies have shown that the isolated components and the crude extracts in different solvents have strong biological activities especially antioxidant, anti-inflammatory,

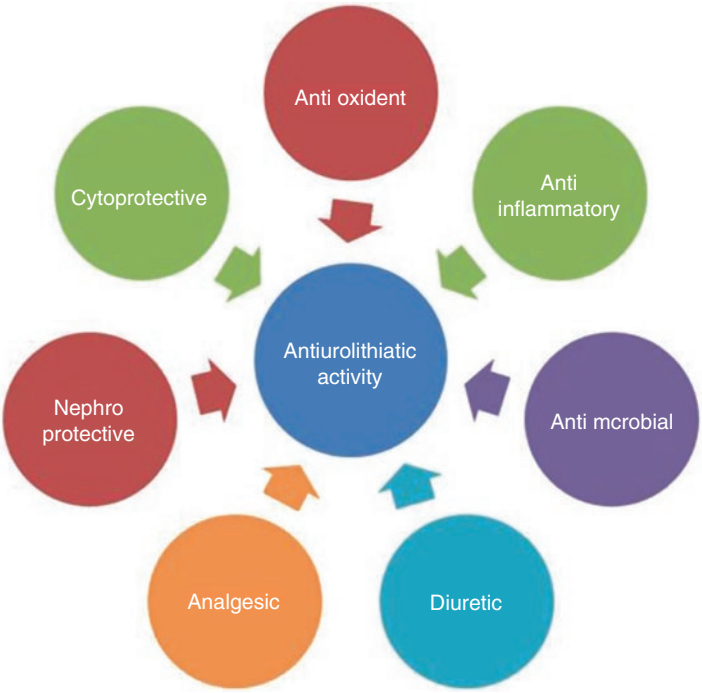

Fig. 16.3 Possible pharmacological actions responsible for antiurolithiatic activity

antimicrobial and diuretic. These activities are consistent with the use of Unani botanicals in the treatment and management of urolithiasis. However, various important issues need to be put across such as in-depth study of chemical structures of Unani botanical-derived compound with molecular and cellular mechanisms. These compounds should be subjected for detailed toxicity studies so that their safety, efficacy and therapeutic activity can be established. These studies may provide a better understanding of the pharmacological effect of Unani medicinal plants and give insight for the development of safe and effective drug in prevention and management of renal stones. Recombinant DNA technology can be used to produce plant proteins and peptides in large quantity with much emphasis on potential toxicity, allergenicity and stability of peptides. Antilithiatic plant proteins will open a new chapter for using plant proteins as therapeutic agents to treat urolithiasis. Preclinical and randomized controlled trials are required to evaluate the health potentials of antilithiatic proteins.

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