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1.1. Drug discovery from natural products

Natural products continue as a source for innovation in drug discovery by playing a significant role in the discovery and understanding of cellular pathways that are an essential component in the drug discovery process. Over the past 75 years, natural product derived compounds have led to the discovery of many drugs to treat human disease. In many cases, natural products provide compounds as clinical/marketed drugs, or as biochemical tools that demonstrate the role of specific pathways in disease and the potential of finding drugs. Numerous reviews have been written that describe the importance of compounds derived from microbes, plants and animal sources to treat human diseases [1, 2]. The 60-75% new drugs in the areas of cancer and infectious disease respectively originate from natural sources.

Since centuries, mankind has relied on natural products as the primary source for medicines. Herbs, bread mold, even leeches were employed to bring relief to the sick and infirm. There was little significant change over much of this time period, however, the last two centuries have brought an explosion of understanding how these natural products are produced and how they interact with other organisms. The last two centuries have seen the isolation of the first commercial drug (morphine), the use of microbial products as medicines (penicillin), and even a use for the lowly leech (the anticoagulant, hirudin).

Even today there are many drugs, available in the markets which are mere mixture of plants and herbal formulations. Various herbal formulations are available for kidney stone disease, like Cystone, Uriflow, Uriflush etc. The constituents of these drugs are either whole plant or an effective part of that plant. The phytochemicals of these plants are yet not fully characterized and still the antilithiatic constituents are unexplored. Therefore, it is important to characterize the active biomolecules of these plants and formulate ways of their artificial synthesis to prevent both the disease and exploitation of herbs.
1.2. *Trachyspermum ammi* (L.) Sprague.

*Trachyspermum ammi*, commonly known as ‘Ajwain’, or ‘Ajowan’ or ‘Bishop’s weed’, is said to be native of Egypt, but is cultivated more extensively throughout India for its seeds, which are used as a spice and in traditional medicine. As Ajowan has a characteristic aromatic smell and pungent taste, leading to its use as a spice in curries. It is employed either alone or in mixture with other spices and condiments. It is also used in pickles, certain types of biscuits, confectionary, beverages and pan mixtures. However, another important use of *Trachyspermum ammi* is medicinal and it is a household remedy for indigestion. The steam-distilled essential oil of the seed is used as a natural commercial source of thymol. Although *Trachyspermum ammi* is also cultivated in the Mediterranean region and South West Asian countries, it is chiefly produced in the Indian states of Madhya Pradesh, Andhra Pradesh, Gujrat, Maharashtra and Uttar Pradesh. The crop is grown in comparative cold but humid weather. Some of the best quality small-seeds varieties are produced around Gwallor, Indore and Ujjain. The seeds and its essential oil are highly valued ingredients in the traditional Unani and Ayurvedic medicines of India [3]

The plant *Trachyspermum ammi*, is an erect branched annual plant upto 90 cm long, cultivated almost throughout India. The stem is 7-9 mm thick near the base and is striated throughout its length. The plant has a tap root system and the stem braches profusely. The aerial parts (leaves) may be either glabrous or pubescent. The leaves are tender, pinnately divided, 24 cm long and 14 cm across at the maximum spread and possess clasping leaf base, shown in figure 1.1. The first pair of leaf segment arises within 3 cm of the base while the second arises at a distance of 8 cm.

![Figure 1.1. Plant of Trachyspermum ammi](image-url)
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*Trachyspermum ammi* is much valued for the aromatic spicy seeds forming on compound umbels, which have been described as being similar in appearance to those of fennel or caraway. The characteristic odor and taste of the seeds is due to the presence of an essential oil. Figure 1.2 shows the image of the seeds of *Trachyspermum ammi*. The seeds of *Trachyspermum ammi* are ovid, aromatic cremocarps, 2-3 mm long, grayish brown in color, mesicarps compressed, with distinct ridges and tubular surface. The seeds easily divide into two one-seeded mericarps. The term ‘fruit’ and seed appear to be used interchangeably in the literature.

![Figure 1.2. Seeds of Trachyspermum ammi](image)

1.2.1. Dietary use of *Trachyspermum ammi*

*Trachyspermum ammi* is widely used as spice in curries due to its aromatic and pungent taste. It is used in pickles, certain confectionary, beverages and pan mixtures. The earliest reference found was by Balbaa et al [4], who cited an Arabic publication of about 1250 AD, in which the use of Nankhawa (*Trachyspermum ammi*) as an appetizer was described. In West Bengal, India, the dried seeds of *Trachyspermum ammi* are chewed as a masticatory [5] and according to Demissew [6] *Trachyspermum ammi* seeds are used in Ethiopia for flavouring a bread and a local alcoholic beverage. Howard et al [7] included *Trachyspermum ammi* in the list of special foods of high nutritive value.
consumed by Napalese lactating women. It is found that the average intake of *Trachyspermum ammi* per capita in South India (in a dietary recall survey of 20 households) was 0.11 g/day, this was 1.15% of the total amount of spices consumed.

### 1.2.2. Therapeutic uses of *Trachyspermum ammi*

*Trachyspermum ammi* is widely used in India and eastern Asia, both in diet and in traditional medicine, the latter use is said to be more common in India. A number of biological actions have been claimed for *Trachyspermum ammi* and investigated in the literature. The major important traditional use of *Trachyspermum ammi* is medicinal; it is a household remedy for indigestion. *Trachyspermum ammi* is also much valued for its antispasmodic, stimulant, tonic and carminative effects, as indicated by various authors.

Balbaa et al [4] stated that previous authors, in about 1250 AD and 1923, had reported the use of *Trachyspermum ammi* for the expulsion of urinary calculi and that the seeds were still used for this purpose. The authors in 1958, also reported on the use of *Trachyspermum ammi* in the treatment of diarrhea, indigestion, atonic dyspepsia, cholera colic and flatulence.

Other reported traditional therapeutic uses of *Trachyspermum ammi* seeds include: galactogogue, stomachic, carminative [8]; expectorant, antiseptic [9]; amoebiasis, antimicrobial; seeds fried in oil and used as a thin soup as a galactogogue [7]; diarrhea, parasiticidal [10]; seeds soaked in lemon juice with Pranus amygdalus given in amenhorroca [11]; bronchitis, a cooling (antipyretic, febrifugal) drink [12] and in typhoid fever [13]. Singh et al [14] reported in a brief review of the pharmacological effects of the spices, note that *Trachyspermum ammi* can be used topically for relieving kidney stones pains.

The seeds are much valued for its antispasmodic, stimulant, tonic and carminative properties. Among other medicinal properties of *Trachyspermum ammi* seeds are its antilithiati and diuretic properties. So far, its diuretic properties have been documented widely in literature and it is actively used in various drug formulations of kidney stone
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treatments [15, 16]. But there are few reports indicating its efficacy towards urolithiasis [17].

Ahsan et al [18] have compared various antilithiatic plants indicated in Ayurvedic system of medicine. Their study reported that Trachyspermum ammi presents 29% protection towards calcium oxalate crystallization. Since, they induced hyperoxaluric conditions by glycolic acid and then evaluated the efficacy of all plants by measuring calcium and oxalate content of kidney tissue, which is not very sensitive approach to evaluate antilithiatic properties, therefore a systematic study focusing on all aspects of urolithiasis and the compositional analysis of Trachyspermum ammi is required to reveal the efficacy of this plant.

1.3 Urolithiasis: the urinary system stone disease

The terms urolithiasis refer to the presence of stone concretions in urinary tract, including kidneys, ureter and urinary bladder (Figure 1.3). Urolithiasis is a problem that has confronted clinicians since the time of Hippocrates, and many family physicians have extensive experience in its clinical management. Even today, kidney stone formation is one of the most painful disorders of urinary tract, affecting 10-15% of the general population world wide.

In recent years, technological advancements have greatly facilitated the diagnosis of kidney stone disease. The management of urolithiasis is also becoming increasingly well defined. Clear indications for urologic referral are based on recognition of the few urgent situations and a solid understanding of the natural history of stone progression.

Kidney stone can vary in size from as small as grains of sand to as large as a golf ball [18]. Kidney stones typically leave the body by passage in the urine stream, and many stones are formed and passed without causing symptoms. If stones grow to sufficient size before passage (on the order of at least 2-3 millimeters) they can cause obstruction of the ureter. The resulting obstruction with dilation or stretching of the upper ureter and renal pelvis as well as spasm of muscle, trying to move the stone, can cause severe episodic pain, most commonly felt in the flank, lower abdomen and groin (a
condition called renal colic). Renal colic can be associated with nausea and vomiting due to the embryological association of the kidneys with the intestinal tract. Hematuria (bloody urine) is commonly present due to damage to the lining of the urinary tract.

![Image of kidney, ureter, and bladder]

**Figure 1.3.** Presence of stones in kidneys, ureter and urinary bladder

Recurrent stone formation can be prevented in most patients by the use of a simplified evaluation, reasonable dietary and fluid recommendations, and directed pharmacologic intervention. Serum studies and 24 hour urine collections are the mainstays of metabolic investigation and usually are warranted in patients with recurrent calculi. Although some stones are the result of inherited conditions, most result from a complex interaction between diet, fluid habits and genetic predisposition.

1.4. **Epidemiology of urolithiasis**

Epidemiology has an important contribution to make in the field of urolithiasis. The prevalence of urolithiasis is approximately 2 to 3 percent in the general population, and the estimated lifetime risk of developing a kidney stone is about 12 percent for white males [19]. Approximately 50 percent of patients with previous urinary calculi have a recurrence within 10 years [20].
Stone disease is two to three times more common in males than in females. It occurs more often in adults than in elderly persons, and more often in elderly persons than in children. Whites are affected more frequently than persons of Asian ethnicity, who are affected more frequently than blacks. In addition, urolithiasis occurs more recurrently in hot, arid areas than in temperate regions.

Decreased fluid intake and consequent urine concentration are among the most important factors influencing stone formation. Certain medications, such as triamterene (Dyrenium), indinavir (Crixivan) and acetazolamide (Diamox), are also associated with urolithiasis. Dietary oxalate is another possible cause, but the role of dietary calcium is less clear, and calcium restriction is no longer universally recommended [21]

1.5. Pathophysiology of urolithiasis

Kidney stones are crystalline mineral deposits that form in the kidney. They develop from microscopic crystals in the loop of Henle, the distal tubule, or the collecting duct and eventually they get enlarged to form visible fragments. The process of stone formation depends on urinary volume, concentrations of calcium ions, phosphate ions, oxalate ions, sodium ions, uric acid ions, urinary pH and the concentrations of natural kidney stone inhibitors (e.g., citrate, magnesium, Tamm-Horsfall, mucoproteins, bikunin etc). High ion levels, low urinary volume, low pH, and low citrate levels favor kidney stone formation. Kidney stones can be due to underlying metabolic conditions, such as renal tubular acidosis [22], Dent's disease [23] and medullary sponge kidney [24]. Some of the risk factors, causing kidney stone formation and their mechanisms of action are listed in table 1.1. Other metabolic causes of kidney stone formation are hypercalciuria, hyperuricosuria, hyperoxaluria and hypocitruria.

1.5.1. Hypercalciuria

Hypercalciuria is defined as excess of calcium excreted in the urine. The content of urinary calcium creating this condition is excretion of calcium above 200 mg in 24 hour urine. Hypercalciuria is the most common metabolic abnormality in patients with calcium stones and results from various mechanisms like, absorptive hypercalciuria (a
condition in which increased absorption of calcium from the gut results in increased circulating calcium, causing increased renal filtration load) and renal hypercalciuria, (a condition in which increased excretion of calcium in the urine results from impaired renal tubular absorption of calcium).

1.5.2. Hyperuricosuria

Uric acid is the end product of purine metabolism and is either derived from exogenous (dietary) sources or produced endogenously during cell turnover. Chronic metabolic acidosis can result in protein metabolism and thus increased excretion of uric acid and formation of kidney stones [25]. Pure uric acid stones are rare but recur frequently.

A low urinary pH below 5.5 is the most common and important factor in uric acid stones. In non-mouricosuric stone disease the primary defect seems to be in the renal excretion of ammonia and is linked to an insulin resistant state [26]. Hyperuricosuria occurs in 10% of patients with calcium stones, where uric acid crystals form the nidus for deposition of calcium and oxalate. A history of gout doubles the risk of kidney stones in men [27].

1.5.3. Hyperoxaluria

Hyperoxaluria is defined as excess of oxalate excreted out in the urine. Excretion of oxalate above of 45 mg/day causes hyperoxaluric conditions. Hyperoxaluric conditions can result from increased intestinal absorption due to ileal disease (Crohn’s disease, ileal bypass) or short bowel syndrome, low calcium intake, or gastrointestinal decolonisation of Oxalobacter formigenes. Increased ingestion of oxalate contributes to about half of the urinary oxalate [28]. Spinach, rhubarb, beets, chocolate, nuts, tea, wheat bran, strawberries, and soya foods are known to increase urinary oxalate concentrations [29]. Vitamin C supplementation may increase urinary oxalate excretion and the risk of calcium oxalate crystallization in patients who form calcium stones [30].
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**Table 1.1.** Risk factors for the development of urinary stone disease.

<table>
<thead>
<tr>
<th><strong>Risk factor</strong></th>
<th><strong>Mechanisms</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bowel disease</td>
<td>Promotes low urine volume; acidic urine depletes available citrate; hyperoxaluria</td>
</tr>
<tr>
<td>Excess dietary meat (including poultry)</td>
<td>Creates acidic urinary milieu, depletes available citrate; promotes hyperuricosuria</td>
</tr>
<tr>
<td>Excess dietary oxalate</td>
<td>Promotes hyperoxaluria</td>
</tr>
<tr>
<td>Excess dietary sodium</td>
<td>Promotes hypercalciuria</td>
</tr>
<tr>
<td>Family history</td>
<td>Genetic predisposition</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Ammonia mishandling; alters pH of urine</td>
</tr>
<tr>
<td>Gout</td>
<td>Promotes hyperuricosuria</td>
</tr>
<tr>
<td>Low urine volume</td>
<td>Allows stone constituents to supersaturate</td>
</tr>
<tr>
<td>Obesity</td>
<td>May promote hypercalciuria; other results similar to excess dietary meat</td>
</tr>
<tr>
<td>Primary hyperparathyroidism</td>
<td>Creates persistent hypercalciuria</td>
</tr>
<tr>
<td>Prolonged immobilization</td>
<td>Bone turnover creates hypercalciuria</td>
</tr>
<tr>
<td>Renal tubular acidosis</td>
<td>Alkaline urine promotes calcium phosphate supersaturation; loss of citrate</td>
</tr>
</tbody>
</table>
Primary hyperoxaluria is an inborn error of metabolism (glycolic aciduria) which is genetically linked cause of hyperoxaluria. In experimental animals, testosterone promotes stone formation by suppressing osteopontin expression in the kidney and increasing urinary oxalate excretion. Oestrogen seems to inhibit stone formation by increasing osteopontin expression in the kidney and decreasing urinary oxalate excretion [31].

1.5.4. Hypocitruria

Hypocitruria is defined as urinary citrate excretion above 250 mg in 24 hours urine. Urinary citrate forms a soluble complex with calcium that inhibits the formation and propagation of crystals. It is a common correctable cause of recurrent pure calcium phosphate or brushite stones. Urinary citrate is mainly derived endogenously through the triarboxylic acid cycle and is excreted by renal tubular cells. Women excrete more citrate and have lower incidence of stone formation than men.

1.6. Diagnosis of urolithiasis

Urolithiasis should always be considered in the differential diagnosis of abdominal pain. The classic presentation of urolithiasis is excruciating unilateral flank or lower abdominal pain of sudden onset that is not related to any precipitating event and is not relieved by postural changes or non-narcotic medications. With the exception of nausea and vomiting secondary to stimulation of the celiac plexus, gastrointestinal symptoms are usually absent. The pain of urinary stone often begins as vague flank pain. Patients frequently dismiss this pain until it evolves into waves of severe pain. It is generally believed that a stone must at least partially obstruct the ureter to cause pain. The pain is commonly referred to the lower abdomen and to the ipsilateral groin. As the stone progresses down the ureter, the pain tends to migrate caudally and medially. The relation ship of site of pain with the location of stone is given in table 1.2. Distal ureteral stones may be manifested by bladder instability, urinary frequency, dysuria and/or pain radiating to the tip of the penis, or the labia or vulva. Increasingly, however, stones are
encountered in asymptomatic patients and are found incidentally on imaging studies or during the evaluation of microhematuria.

Table 1.2. Relationship of stone location to common symptoms.

<table>
<thead>
<tr>
<th>Stone location</th>
<th>Common symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>Vague flank pain, hematuria</td>
</tr>
<tr>
<td>Proximal ureter</td>
<td>Renal colic, flank pain, upper abdominal pain</td>
</tr>
<tr>
<td>Middle section of ureter</td>
<td>Renal colic, anterior abdominal pain, flank pain</td>
</tr>
<tr>
<td>Distal ureter</td>
<td>Renal colic, dysuria, urinary frequency, anterior abdominal pain, flank pain</td>
</tr>
</tbody>
</table>

Symptoms similar to those of urinary stones can be caused by non-calculus conditions. In women, gynecologic processes like ovarian torsion, ovarian cyst and ectopic pregnancy have similar symptoms. In men, symptoms of testicular processes, such as a tumor, epididymitis or prostatitis, may mimic the symptoms of distal ureteral stones. Other general causes of abdominal pain, such as appendicitis, cholecystitis, diverticulitis, colitis, constipation, hernias or even arterial aneurysms, may elicit similar discomfort. Symptoms mimicking those of urolithiasis also occur with urologic lesions such as congenital uretero-pelvic junction obstruction, renal or ureteral tumors, and other causes of ureteral obstruction.
Many family physicians have had experience with patients whom they suspect of having factitious stones. Frequently, these patients claim to be "allergic" to intravenous contrast media [32]. Non-contrast helical computed tomography (CT) is a relatively new modality with the capability to exclude stones in such problem patients.

The diagnosis of urinary tract stones begins with a focused history. Key elements include past or family history of stones, duration and evolution of symptoms, and signs or symptoms of sepsis. The physical examination is often more valuable for ruling out non-
urologic disease. Urinalysis should be performed in all patients with suspected stones. Aside from the typical microhematuria, important findings to note are the urine pH and the presence of crystals, which may help to identify the stone composition. Patients with uric acid stones usually excrete acidic urine and those patients in which the cause of stone formation is infection, have alkaline urine. Identification of bacteria is important in planning therapy and a urine culture should be routinely performed.

Because of the various presentations of urolithiasis and its broad differential diagnosis, an organized diagnostic approach is useful (as shown in figure 1.4). Symptomatic stones essentially cause a severe abdominal pain. Although urinary stones can be suspected in a patient based on the history and physical examination, but diagnostic imaging is essential to confirm or exclude the presence of urinary calculi. Several imaging modalities are available and their advantages and limitations are discussed below.

1.6.1. Abdominal ultrasonography

Abdominal ultrasonography has limited use in the diagnosis and management of urolithiasis. Although, ultrasonography is readily available, quickly performed and sensitive to all urinary stones, it is virtually blind to ureteral stones (sensitivity: 19%). However, if a ureteral stone is visualized by ultrasound, the finding is reliable (specificity: 97%). The ultrasound examination is highly sensitive to hydronephrosis, which may be a manifestation of ureteral obstruction, but it is frequently limited in defining the level or nature of obstruction. It is also useful in assessing renal parenchymal processes, which may mimic kidney stone. Abdominal ultrasonography is the preferred imaging modality for the evaluation of gynecologic pain, which is more common than urolithiasis in women of childbearing age.

1.6.2. Plain-film radiography

Plain-film radiography of the kidneys, ureters and bladder (KUB) may be sufficient to document the size and location of radiopaque urinary stones. Stones that contain calcium, such as calcium oxalate and calcium phosphate stones, are easiest to
detect by radiography. Less radiopaque calculi, such as pure uric acid stones and stones composed mainly of cystine or magnesium ammonium phosphate, may be difficult, if not impossible, to detect on plain-film radiographs. Unfortunately, even radiopaque calculi are frequently obscured by stool or bowel gas, and ureteral stones overlying the bony pelvis or transverse processes of vertebrae are particularly difficult to identify. Furthermore, non-urologic radio-opacities, such as calcified mesenteric lymph nodes, gallstones, stool and phleboliths (calcified pelvic veins) may be misinterpreted as stones. Although 90% of urinary stones have historically been considered to be radiopaque, the sensitivity and specificity of KUB radiography alone remain poor (sensitivity: 45% to 59%; specificity: 71% to 77%). KUB radiographs are useful in the initial evaluation of patients with known stone disease and in following the course of patients with known radio-opaque stones.

1.6.3. Intravenous pyelography

Intravenous pyelography has been considered the standard imaging modality for urinary tract calculi. The intravenous pyelogram provides useful information about the stone (size, location, radiodensity) and its environment (calyceal anatomy, degree of obstruction), as well as the contralateral renal unit (function, anomalies). Intravenous pyelography is widely available, and its interpretation is well standardized. With this imaging modality, ureteral calculi can be easily distinguished from nonurologic radiopacities. The accuracy of intravenous pyelography can be maximized with proper bowel preparation, and the adverse renal effects of contrast media may be minimized by ensuring that the patient is well hydrated.

Unfortunately, these preparatory steps require time and often cannot be accomplished when a patient presents in an emergency situation. Compared with abdominal ultrasonography and KUB radiography, intravenous pyelography has greater sensitivity (64% to 87%) and specificity (92% to 94%) for the detection of renal calculi. However, the intravenous pyelogram can be confusing in the presence of non-obstructing radiolucent stones, which may not always generate a “filling defect [33, 34].
Furthermore, in patients with high-grade obstruction, even prolonged re-imaging at 12 to 24 hours may not demonstrate the level of obstruction because of inadequate concentration of the contrast medium.

1.6.4. Non-contrast helical CT

Non-contrast helical CT is being used increasingly in the initial assessment of renal colic [35, 36]. This imaging modality is fast and accurate, and it readily identifies all stone types in all locations. Its sensitivity (95 to 100 percent) and specificity (94 to 96 percent) suggest that it may definitively exclude stones in patients with abdominal pain [37, 38, 39, 40] Associated signs, such as renal enlargement, perinephric or periureteral inflammation or “stranding,” and distension of the collecting system or ureter, are sensitive indicators of the degree of ureteral obstruction [41].

Hounsfield density of calculi may be used to distinguish cystine and uric acid stones from calcium-bearing stones and is capable of further subtyping the calcium stones into calcium phosphate, calcium oxalate monohydrate and calcium oxalate dihydrate stones [42]. Non-contrast helical CT is also useful in diagnosing nonurologic causes of abdominal pain, such as abdominal aortic aneurysms and cholelithiasis. The estimated sizes of renal calculi determined using this imaging technique varies slightly from those obtained with KUB radiography.

Non-contrast helical CT is generally more expensive than intravenous pyelography, but the increased cost is certainly balanced by more definitive, faster diagnosis. In one study, [43] the cost of non-contrast helical CT was reported as $600 compared with $400 for intravenous pyelography; cost obviously varies from institution to institution and by accounting methods.

In the future, non-contrast helical CT may become the imaging technique of choice and the standard of care. Its emergence as the definitive initial imaging modality for urolithiasis may allow intravenous pyelography to be reserved for therapeutic planning in complex stone cases.
1.7. Features of kidney stones

Kidney stones are classified based on what they are made up of or their composition. There are five basic types of kidney stones but most stones are either calcium stones or uric acid stones. Various types of kidney stones are mentioned below.

1.7.1. Calcium stones

The most common type of kidney stone (75-80%) is made up of calcium binding principally to oxalates but to phosphates as well. Both oxalates and phosphates are found in the food we eat, but unlike phosphates, oxalates not reported to be of any use yet. Both calcium oxalate and calcium phosphate calculi are described below.

1.7.1.1. Calcium oxalate stones

Calcium oxalate stones are the most common type of urinary calculi and can exist in monohydrate and dihydrate forms, with or without phosphate. High phosphate content may be associated with higher recurrence rates [44]. Calcium oxalate stones are radiopaque and usually visible on plain film radiography or noncontrast CT. The causes of calcium oxalate stones and their mechanisms are listed in Table 1.3. Hypercalciuria (i.e., more than 250mg per 24 hours) is the most common metabolic abnormality associated with these calculi, followed by hypocitraturia (i.e., less than 450 mg per 24 hours), which involves a deficiency of the naturally occurring stone inhibitor citrate. The cause of hypocitraturia often is idiopathic, although high dietary acid loads (e.g., from excessive meat intake) and dehydration can exacerbate this condition. Other causes of calcium oxalate stones include hyperoxaluria (i.e., more than 45 mg per 24 hours) and hyperuricosuria (i.e., more than 800 mg per 24 hours).

1.7.1.2. Calcium phosphate stones

Calculi that consist predominantly of calcium phosphate occur more often in women than in men. They are often associated with acidification disorders such as renal tubular acidosis [45]. Less common etiologies include primary hyperparathyroidism, excessive alkalinization, and sarcoidosis. Renal tubular acidosis is associated with hypercalciuria and hypocitraturia.
### Table 1.3. Common causes of calcium oxalate stones

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Possible mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercalciuria (more than 250 mg per 24 hours)</td>
<td></td>
</tr>
<tr>
<td>Absorptive hypercalciuria</td>
<td>Increased intestinal absorption of calcium</td>
</tr>
<tr>
<td>Idiopathic hypercalciuria</td>
<td>Inherited trait</td>
</tr>
<tr>
<td>Primary hyperparathyroidism</td>
<td>Increased bone demineralization or increased intestinal calcium absorption</td>
</tr>
<tr>
<td>Renal hypercalciuria</td>
<td>Renal leak of calcium</td>
</tr>
<tr>
<td>Hypoxaluria (more than 45 mg per 24 hours)</td>
<td></td>
</tr>
<tr>
<td>Enteric hypoxaluria</td>
<td>Malabsorption from any cause with increased urinary oxalate to complex with calcium</td>
</tr>
<tr>
<td>Primary hypoxaluria</td>
<td>Metabolic error with high level of oxalate production and urinary excretion</td>
</tr>
<tr>
<td>Hyperuricosuria (more than 800 mg per 24 hours)</td>
<td>Increased uric acid promotes calcium oxalate crystallization via the formation of nuclei</td>
</tr>
<tr>
<td>Hypocitraturia (less than 450 mg per 24 hours)</td>
<td>Idiopathic; renal tubular acidosis (types 1, 2, and 4)</td>
</tr>
</tbody>
</table>
1.7.2. Uric acid stones

Uric acid stones may consist of uric acid only, or they also may contain calcium [46]. Uric acid is a by-product of ingested or endogenous purine metabolism and is excreted in the urine primarily in insoluble form. The primary cause of uric acid stones is a urinary pH below the pKa for uric acid i.e. 5.5. Other predisposing conditions include gout, insulin resistant states, and end-ileostomies. Men with gout have a twofold risk of having a uric acid calculus [47]. In general, these patients excrete excessive uric acid (although some have normouricosuria) and have low urinary pH and urine volumes. Excess ingestion of animal meat protein (i.e. meat of all types, including poultry) can be detected by measuring urinary sulfate levels. Radiographic imaging can be difficult because pure uric acid calculi typically are radiolucent. They are, however, readily apparent on noncontrast CT.

1.7.3. Struvite stones

Struvite stones, also known as infection or triple-phosphate stones, consist of magnesium, ammonium and calcium phosphate. They occur more often in women than in men and are the leading cause of staghorn calculi. Neurogenic bladders and foreign bodies in the urinary tract also predispose patients to struvite calculi. Recurrent urinary tract infections with urea-splitting organisms (e.g., Proteus mirabilis, Ureaplasma urealyticum, Klebsiella pneumoniae) result in alkalinization of urine and the addition of ammonium to the milieu [48]. Struvite stones are usually radiopaque on standard radiographic imaging but may be quite faint. Patients with struvite calculi may present with flank pain and may have signs of systemic infection.

1.7.4. Cystine stones

Patients with cystine calculi have an autosomal recessive disorder of dibasic amino acid transport leading to decreased cystine resorption in the kidney. Only homozygote patients form cystine calculi and often present with stones during childhood. Calculi may be pure cystine or may be mixed with calcium oxalate. Cystine is poorly soluble at normal urinary pH and will readily form stones when levels rise above a
concentration of 250 mg per litre. Pure cystine stones are yellow and radiolucent or faintly radiopaque. A urinary cystine level of more than 250 mg per 24 hours (1,040 μmol per day) is diagnostic parameter for this disorder.

1.8. Present day management strategies for urolithiasis

The management of urolithiasis is divided into management of emergency situations and stone management.

1.8.1. Emergency situation management strategy

The first step is to identify patients who require emergency urologic consultation. For example, sepsis in conjunction with an obstructing stone represents a true emergency. In patients with sepsis, adequate drainage of the system must be established with all possible speed by means of percutaneous nephrostomy or retrograde ureteral stent insertion. Other emergency conditions are anuria and acute renal failure secondary to bilateral obstruction, or unilateral obstruction in a patient with a solitary functioning kidney.

Hospital admission may be required for patients who are unable to maintain oral intake because of refractory nausea, debilitated medical status or extremes of age, or for patients with severe pain that does not respond to narcotic therapy. Placement of a retrograde ureteral stent or percutaneous nephrostomy tube may be a useful temporary measure in patients with refractory symptoms. For all other patients, ambulatory management of kidney stone should be adequate. The basis of ambulatory management is adequate analgesia, timely urologic consultation and close follow-up.

Numerous medical strategies have been attempted to control urolithiasis, which can be attributed to ureteral spasm. Although narcotics such as codeine, morphine and meperidine (Demerol) are effective in suppressing pain, they do nothing to treat its underlying cause, and they have the side effects of dependence and disorientation. As a result of combined anti-inflammatory and spasmylytic effects, non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, diclofenac (Voltaren) and ibuprofen (e.g., Motrin) can be effective in managing the pain of kidney stone. Of these agents, ketorolac
(Toradol) merits special mention. In one emergency department study, the narcotic-like analgesic effects of this agent were superior to the effects of meperidine [49]. Unfortunately, the antiplatelet effects of NSAIDs (including ketorolac) are its contraindication. Therefore, NSAIDs administration is restricted to patients undergoing extracorporeal shock wave lithotripsy, because of the increased risk of perinephric bleeding [50, 51]

The cyclooxygenase-2 inhibitors, a new class of NSAIDs, may prove to be effective agents in the management of kidney stones. Theoretically, these drugs do not impair platelet function. To date, however, there have been no reports in literature, of their use in patients with kidney stones patients. At present, an effective approach for a kidney stone outpatient is to use both an oral narcotic drug and an oral NSAID.

1.8.2. Stone management strategy

After emergency situations have been ruled out and adequate analgesia has been achieved, the next step is to formulate a strategy for managing the stone. Clinical experience with urolithiasis has been refined with statistical analysis to provide sound principles for definitive management [52]. The likelihood that a ureteral stone will pass appears to be determined by its size (i.e., greatest diameter). Stones less than 5 mm in size should be given an opportunity to pass. Patients can be advised that stones less than 4 mm in size generally pass within one to two weeks. With stones of this size, 80% of patients require no intervention beyond analgesia.

Patients with a radiopaque ureteral stone who elect a conservative approach should be advised to have regular follow-up of kidney, ureter and bladder radiographs at one to two week intervals. They should also strain their urine to capture stones or stone fragments, because stone composition provides important information for the prevention of future stones. Patients should be cautioned to seek immediate medical attention if they develop signs of sepsis. The principal message should be that medical surveillance must be continued until stone passage is documented. Although unlikely with small stones, asymptomatic complete ureteral obstruction may destroy renal function in as less as six to eight weeks.
As stones increase in size beyond 4 mm, the need for urologic intervention increases exponentially. Referral to urologist is indicated for patients with a stone greater than 5 mm in size. Referral is also indicated for patients with a ureteral stone that has not passed after two to four weeks of observation. The complication rate for ureteral calculi has been reported to almost triple (to 20%) when symptomatic stones are left untreated beyond four weeks [53]. Renal stones, which are generally asymptomatic, may be followed conservatively. However, patients can be advised that about 50 percent of small kidney stones become symptomatic within five years of detection [54].

Medical prophylaxis of recurrent urinary stones includes generalized recommendations and specific directed therapy when appropriate. As noted previously, patients with recurrent episodes warrant a more aggressive approach. Evidence which shows that increased water intake reduces the risk of recurrence of urinary stones and prolongs the average interval between recurrences. A target of 2.1 qt (2 L) of urine production per day generally is recommended [55]. The present scenario of stone management includes medicinal management and surgical management of kidney stone.

1.8.2.1. Medicinal management: the expulsive therapy

Spontaneous passage of urinary stones of about 4 mm in diameter may occur in as many as 98% of patients, although the stone may take 40 days or more to pass [52]. Several mathematical models have been developed that predict the likelihood of spontaneous stone passage with high accuracy [56, 57, 58]. However, prolonged partial obstruction (more than 6 weeks), the persistence of pain, or the presence of urinary infection, mandate active intervention i.e. ureteral stenting, extracorporeal shock wave lithotripsy (ESWL) or percutaneous nephrolithotomy [59, 60]. Medical expulsive therapy has been recommended to promote stone passage and reduce the need for ESWL or minimally invasive surgery. There are several classes of drugs with different mechanism of action that promote expulsion of stones (Table 1.4).
Table 1.4. Summary of clinical trials of drugs promoting stone expulsion

<table>
<thead>
<tr>
<th>Therapy</th>
<th>No. of Patients</th>
<th>Mean stone size</th>
<th>Stone location</th>
<th>Stone passage rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diclofenac sodium</td>
<td>80</td>
<td>&lt;5.0</td>
<td>Any</td>
<td>57.5</td>
</tr>
<tr>
<td>Nifedipine + methylprednisolone vs placebo</td>
<td>43</td>
<td>6.7 vs 6.8</td>
<td>Any</td>
<td>87 vs 65</td>
</tr>
<tr>
<td>+ methylprednisolone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nifedipine + deflazacort vs watchful waiting</td>
<td>48</td>
<td>5.8 vs 5.5</td>
<td>Distal</td>
<td>79 vs 35</td>
</tr>
<tr>
<td>Nifedipine + prednisolone vs prednisolone</td>
<td>25</td>
<td>12 vs 12.8</td>
<td>Any</td>
<td>81 vs 68</td>
</tr>
<tr>
<td>Tamsulosin vs control</td>
<td>51</td>
<td>&lt;10</td>
<td>Distal</td>
<td>80 vs 63</td>
</tr>
<tr>
<td>Tamsulosin + deflazacort vs nifedipine +</td>
<td>28 vs 30</td>
<td>4.7 vs 5.4</td>
<td>Distal</td>
<td>85 vs 80 vs 43</td>
</tr>
<tr>
<td>deflazacort vs control</td>
<td>vs 28</td>
<td>vs 5.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamsulosin vs deflazacort</td>
<td>33 vs 24</td>
<td>6.0 vs 5.8</td>
<td>Distal</td>
<td>60 vs 37.5</td>
</tr>
</tbody>
</table>

A few studies suggest the use of corticosteroids such as hydroxyprogesterone, along with NSAIDs to improve stone passage. The former can promote ureteral relaxation and dilation. And the later can stimulate stone expulsion by reducing inflammation and edema, relaxing pelviureteral wall smooth muscle and reducing
intrapelvic pressure [61, 62, 63]. However, the utility of these classes of drugs remains uncertain in expulsive stone therapy and more studies are necessary to validate their effectiveness.

Calcium-channel blockers such nifedipine represent a valid and well established pharmacologic treatment for urolithiasis owing to their spasmylytic action on the ureter. In both animal and human ureters, nifedipine eliminates the fast uncoordinated component of ureteral smooth muscle contraction, leaving unmodified the slower peristaltic activity [64].

Indeed several authors have demonstrated enhanced stone passage in patients treated with nifedipine (30mg/day slow release for 20-30 days) plus steroid as an antiedema agent (25mg/day of methylprednisolone or 30mg/day of def;azacort for 10 days). A higher stone expulsion rate, shorter expulsion time and reduced need for analgesia with an associated good tolerability and safety have been shown in several trails [65, 66]. Caution must be used when administering nifedipine to patients with cardiovascular disease because of the risk of serious side effects such as hypotension or palpitation. Minor side effects reported with nifedipine include headache and asthenia.

The presence of alpha and beta adrenergic receptors has been demonstrated in human ureters [67]. Alpha-1 receptors, particularly subtype alpha-1d, are present in high density in lower ureteral segment and may play an important role in lower ureteral physiology through an effect on detrusor and ureteral smooth muscle contraction [68]. On the basis of these findings, the use of alpha-1 for accelerating the expulsion of lower ureteral stones was tested.

Several investigators have shown utility of alpha-1 blockers and their spasmylytic action in the active expulsion of stones from the distal ureter with a low incidence of side effects such as hypotension and asthenia [69, 70]. In two recent randomized controlled trials comparing tamsulosin and nifedipine combined with corticosteroids with placebo for lower ureteral stones, a higher stone expulsion rate and reduced need for analgesia was demonstrated for both the drugs compared with placebo.
[66]. However, tamsulosin (0.4mg.day for 4 weeks) was associated with a shorter time to stone expulsion and less need for hospitalization [66]. In the expulsion therapy, an important factor is edema of ureteral wall caused by stone irritation. It represents a cause of arrest of stone passage with consequent obstruction. Corticosteroids, especially deflazacort, are frequently used as an anti-edema agent in association with calcium channel blockers and alpha blockers in order to promote stone expulsion [64, 66]. In general, corticosteroids are well tolerated if used for short periods of time.

The medicinal management for a specific stone type is different. Some specific treatment for specific type of stones is mentioned below. The algorithm for medicinal management of recurrent calculi is given in figure 1.5.

1.8.2.1.1. Calcium oxalate stone treatment

Depending on the cause of calcium oxalate stone, the available medication is different. As for hypercalciuria, thiazide, diuretic and potassium citrate drugs are prescribed. There are no specific medications for hyperoxaluria. Medical treatment consists of increasing calcium intake (particularly with meals) to control enteric hyperoxaluria is adopted. Additionally, decreasing the intake of oxalates contained in foods such as spinach, rhubarb, beets, chocolate, nuts, tea, strawberries, soy foods, and wheat bran may be beneficial [28]. Calcium oxalate calculi not associated with an obvious laboratory abnormality can be treated empirically with oral potassium citrate (Urocit-K, 30 to 60 mEq per day) or sodium citrate (Bicitra) to increase urine pH and levels of urinary citrate [71, 72].

1.8.2.1.2. Calcium phosphate stone treatment

Medical treatment of these stones consists of replenishing urinary citrate to prevent new stone formation and delay growth of existing stones. Care must be taken to avoid excessive alkalinization, because high urinary pH can increase the urinary supersaturation of calcium phosphate salts. If hypercalciuria persists, addition of a thiazide diuretic is indicated [73].
Medical Management of Nephrolithiasis

Stone episode resolved?

Yes
- Obtain history: Number of previous episodes, onset of previous episode, bowel disease, gout, diabetes, medications, family history
- Serum studies: 24-hour urine studies
- Cystinuria
- Urinary acid calculi
- Hypercalciemia
- Hyperparathyroidism
- Hyperuricosuric
- Allopurinol
- Add sodium citrate
- Treat based on findings
- Add potassium citrate if patient has low urine citrate levels

No
- Consider lower sodium intake (all types)
- Uncomplicated calcium stone disease (i.e., normocalciemia, no bowel disease, no urinary tract infection)
- No calcium
- Hypercalcemia
- Hyperparathyroidism
- Treat with thiazide diuretics
- Add potassium citrate if patient has low urine citrate levels

Conservative management: Increase urine output to 2.1 qt.

Previous episode?

Conservative management: Increase urine output to 2.1 qt.

Consider lower sodium intake (all types)

Uncomplicated calcium stone disease (i.e., normocalciemia, no bowel disease, no urinary tract infection)

No calcium

Hypercalcemia

Hyperparathyroidism

Treat with thiazide diuretics

Add potassium citrate if patient has low urine citrate levels

Figure 1.5. Algorithm for medicinal management of recurrent kidney stones
1.8.2.1.3. Struvite stone treatment

There is good evidence that failure to treat struvite stones can lead to an increased risk of renal loss, sepsis, and death [74, 75]. However, if the patient is febrile or presents signs of systemic infection, surgical manipulation should be delayed until antibiotic treatment has been administered and the patient has been out of fever for at least 48 hours. After surgical intervention, medical therapy should focus on preventing recurrent urinary tract infections. Retained residual fragments increase the risk of recurrent urinary tract infection and future stone occurrence. Acetohydroxamic acid (Lithostat) is an irreversible inhibitor of urease and can prevent the crystallization of struvite stones [76]. However, because of side effects (including deep venous thrombosis), it is generally reserved for use in patients who cannot tolerate surgical intervention [77].

1.8.2.1.4. Uric acid stone treatment

The treatment of uric acid stones involves correction of urinary pH. Potassium citrate at a dosage of 30 to 60 mg per day will raise the urinary pH to greater than 5.5 (6.5 to 7 is ideal) [78]. Allopurinol (Zyloprim) at a dosage of 300 mg daily can be added in patients with hyperuricemia.

1.8.2.1.5. Cystine stone treatment

Dietary manipulation with a low-methionine diet is difficult and rarely successful. Hydration and administration of urinary alkalinizing agents such as potassium citrate are mainstays of therapy. However, it is often difficult to achieve adequate alkalinization with oral agents. If these measures are not effective, administration of cystine binders such as penicillamine (Cuprimine) and tiopronin (Thiola) can help prevent cystine calculi. Although these agents are effective, they can cause significant side effects such as gastrointestinal distress, rheumatologic symptoms, mental status changes and skin rashes [79]. After the initial stone episode has resolved, patients should be counseled about prevention of recurrences. A basic evaluation should include a thorough history, including age at onset, frequency and number of previous calculi, and any previous medical or surgical interventions.
Additional information for evaluating prevention measures of kidney stones should include an evaluation of fluid and dietary habits and a history of predisposing conditions such as bowel disease, gout and a family history of urinary stones. Serum studies should include electrolyte, calcium, phosphate, uric acid and intact parathyroid hormone levels. A more thorough evaluation has been advocated for patients who have had more than one stone episode.

In the patients have recurrence of stone episodes, the expense of additional laboratory tests and pharmacotherapy likely is less than the expense of repeat emergency department visits and surgical management [80, 81]. An expanded evaluation includes two 24-hour urine collections to determine urine volume, pH, and calcium, creatinine, sodium, phosphate, oxalate, citrate, uric acid, and cystine levels. Crystallographic analysis of retrieved calculus remnants can help identify the underlying etiology and may obviate a complete metabolic evaluation. Medical prophylaxis of recurrent urinary calculi includes generalized recommendations and specific directed therapy when appropriate. As noted previously, patients with recurrent episodes warrant a more aggressive approach.

1.8.2.2. Surgical management

Surgery is only an option when the stone is a size or shape that will prevent its passage and is blocking the flow of urine or when it is causing damage to the kidney or another part of the urinary tract. Recovery time is longest with open surgery. Today, treatment for these stones is greatly improved and in many options do not require major surgery and the recovery time is also reduced. Such treatments include ureteroscopic, percutaneous nephrolithotomy and extracorporeal shockwave lithotripsy.

The surgical procedure of ureteroscopy has replaced open surgery for the majority of kidney stones. For ureteroscopic stone extraction, the urologist looks into the ureter with a small (1/8 inch diameter) telescope to visualize the stone. Once the stone is located, it can either be removed intact via a basket or grasper or it may be broken/fractured and then removed in pieces. This procedure is commonly performed in conjunction with intracorporeal lithotripsy. Percutaneous nephrolithotomy is a procedure
used to remove large stones from the kidney. Although these stones previously were treated with open surgery, even large stones can now be extracted through a 1 inch incision. The procedure begins by placing a percutaneous nephrostomy (a small tube) through the flank and directly into the kidney. Once the tube is placed, the urologist will enlarge the opening and look into the kidney with a small telescope (approximately 0.4 inches in diameter). When the large stone is visualized, it can be fragmented with a laser or ultrasound device and removed in pieces.

Originally developed in the 1980's, shock wave therapy is now a standard method of treating some stones in the kidney and ureter. In general, shock wave therapy is effective for stones up to 1.5 - 2.0 cm in size. Stones larger than 2.0 cm are unlikely to be treated effectively with shock wave therapy. Only stones which can be visualized with standard x-rays can be treated with most lithotripters. Lithotripsy is performed on an outpatient basis and usually takes about an hour. The patient is placed in the lithotripter and the stone is localized by x-ray guidance. An anesthetic is administered and 3,000 to 4,000 shocks are delivered to the stone depending upon its location. Stones within the kidney and upper ureter have a high success rate when treated with lithotripsy. Stones in the lower ureter are more difficult to visualize and treat with lithotripsy and may be better treated with ureteroscopy.

1.9. Drawbacks of Current Treatments

The extracorporeal shock waves lithotripsy is fundamental in the treatment of lithiasis. However, there are evidences that it can produce renal damage [82]. High-energy shock waves (HESW) when applied to rat did not inhibit the animal growth but caused transitory histological lesion in spleen (proliferative changes in the red pulp) and in liver (cloudy swelling of hepatocytes) [83]. Shockwaves can enhance metastasis of tumors and this effect is attributable to cavitations. It has been reported that extracorporeal shock wave lithotripsy also leads to reduced sperm concentration and motility in men [84]. The effects of ESWL, on patients undergoing renal stone treatment have been studied using activities of glucose-6-phosphate dehydrogenase, superoxide dismutase, catalase and levels of malondialdehyde in the erythrocyte haemolysate. Recent
study revealed that ESWL can induce erythrocyte lipid peroxidation and antioxidative defense mechanism may be transiently impaired by it [85]. A case study also shows an unusual complication like rupture of the kidney observed after extracorporeal shock wave lithotripsy [86].

Although some oral medications have positive effects, they are not effective in all patients. Oral medicine has certain side effects also as describes in previous section. In addition, oral citrate is one of the most widely used medical therapies for preventing urinary stone disease. It exerts its preventive effect through increasing urinary pH, decreasing Tamm-Horsfall protein aggregation and decreasing crystal adhesion to tubular cells [87]. However, this drug is not tolerated by all patients and some patients are still active stone formers during this therapy [88].

Due to the adverse effects of these drugs alternative treatment modalities composed of herbal remedies have been the mainstay of medical therapy for thousands of years, especially in Eastern civilizations. Although it is believed that the resurgence of interest in phytotreatment became popular in the second half of the 19th century in Western countries, this complementary medical therapy was widely used in Europe much before that date.

1.10. Calcium oxalate crystals in plants

Many plants produce calcium oxalate as crystalline deposits [89, 90], which can account for greater than 85% of the dry weight of some plant organs. The formation of CaOx is an essential process in many species, and more than 90% of tissue calcium can be tied up as this compound [91]. CaOx crystals often occur within the vacuole of crystal idioblasts, specialized cells that generally encompass less than 1% to 2% of the total cells of the calcium accumulating tissue.

Because calcium oxalate formation is the end result of a mechanism for controlling calcium at the tissue and organ levels in the plant [92, 93], cells producing the crystals are considered to be high-capacity calcium sinks. Because crystal idioblasts perform a unique complex function of importance to the general physiology of the plant,
and they commonly occur as single cells scattered among other tissues, we have referred to them as single-celled organs [94].

**1.10.1. Structure and systematic distribution of calcium oxalate crystals in plants**

Calcium oxalate crystals may form in any organ or tissue within plants. For example, crystals occur in roots, stems, leaves, flowers, fruits, and seeds [95] and within epidermal [96], ground, and vascular [97] tissues. Calcium oxalate often forms in idioblasts cells that develop in isolation with structure or content distinct from surrounding cells [98]. In other instances, crystals may develop in defined groups of cells, as in files of bundle sheath cells [99], for example, or in a single layer of the seed coat [100]. Less often, entire tissues such as endosperm [101] or leaf epidermis [102] accumulate calcium oxalate in every cell or in a majority of cells.

Plant crystals display an astonishing variety of morphologies, most of which conform to one of the following categories defined by botanists [103].

- Prisms, consisting of simple regular prismatic shapes
- Druses, which are spherical aggregates of crystals, shown in figure 1.6A.
- Raphides, acicular crystals that form in bundles, shown in figure 1.6B
- Styloids, acicular crystals that forms singly, shown in figure 1.6C and 1.6D.
- Crystal sand, small tetrahedral crystals that form in clusters.

Calcium oxalate exists in two chemical forms, calcium oxalate monohydrate (COM) and calcium oxalate dehydrate (COD), and both of these forms occur in plants as well as animals [104, 105]. COM is thermodynamically more stable form, and therefore it is usually predominant in both animals and plants. The observed morphologies in figure 1.6, represent elaborations and modifications of basic crystal structure for either the monohydrate or dihydrate form.
Figure 1.6. Photomicrograph of three main type of crystals present in plants. A: showing druses and styloids (arrowhead); B: Raphides projecting into air space; C & D: Styloids into intracellular spaces; E & F: Idioblasts containing druses. [106]
1.10.2. Matrix of calcium oxalate crystals

Webb and Arnott [100] showed that grape druse crystals have a non-mineralized core material of unknown but presumably organic composition, and Webb et al. [107] demonstrated that a complex organic matrix was present within the vacuole of grape raphide idioblasts. This matrix was found to possess the ability to facilitate crystal formation. When isolated calcium oxalate crystals from plants are treated with EDTA, the calcium oxalate is partially or completely dissolved, but non-mineral matrix remains, which can be easily observed with TEM.

As shown in Figure 1.7, this material retains the shape of the original crystal, thus, it is referred as crystal matrix ghost. The crystal matrix ghost is made up of interconnected macromolecules complex which impart it flexibility, such that bending it at a 90° angle. Additionally, the crystal ghost matrix remains intact even after preparing it for TEM analysis, further suggests its flexibility.

Even after EDTA treatment, the central region of these crystals does not de-mineralize completely and small block of crystal remains (Figure 1.7A). Partial dissolution with EDTA can also leave behind small “plates” of calcium oxalate along the matrix (Figure 1.7B). It is also observed that if a crystal matrix ghost is incubated with calcium and oxalate, a crystal forms with essentially the same shape as the ghost, although often the surfaces are rough or have micro-crystals projecting from them. The druse crystals also have a central core of material [93], and after dissolution of the mineral with EDTA, this core material can also initiate crystallization, although the crystal morphology is very irregular. Micro-autoradiography of crystals or crystal matrix exposed to radioactive calcium or oxalate further demonstrates the ability of the matrix to bind these radioactive ions.

Recently, Bouropoulos et al. [108] found that crystals of tomato and tobacco contain macromolecules that can promote CaOx nucleation. Macromolecular matrix materials can hold important implications with respect to crystal morphology and, as pointed out by Arnott and Webb [109], crystal stability.
Figure 1.7. CaOx crystals having a non mineral matrix with an affinity for Ca and oxalate. A: EDTA removes most of the Ca oxalate but leaves a flexible matrix “ghost” in the shape of the original crystal. Note that the middle part of the crystal (arrow) has not been dissolved; B: Higher magnification of the crystal matrix. Some Ca oxalate (arrows) is still present along the matrix ghost [110].

Most calcified tissues in animal systems undergoing controlled mineralization have been found to have an organic matrix associated with them [111], which includes various classes of proteins shown in vitro to be able to control crystal growth and morphology. Such proteins have been found to be integrated into the structure of biominerals of invertebrate organisms such as sponge spicules [112], mollusk shells (113), and sea urchin spines [114], and also in CaOx kidney stones that form in humans [Homo sapiens; 115]. Although macromolecules appear to be involved in nucleation and modifying growth patterns [116, 117], they may also have inhibitory effects as observed in the case of calcium oxalate in the urinary tract [118, 119].

It is interesting to note that the acidic proteins from animal matrix have some physical properties similar to the matrix protein of plants crystals, such as poor solubility
of some of the animal matrix proteins, a tendency to aggregate, and poor staining on SDS-PAGE [116]. It is hypothesized by Xingxiang Li [110] that the proteins isolated from plant calcium oxalate crystals have a similar function to some of the animal matrix proteins in terms of affecting crystal growth. They suggested that crystal matrix protein has calcium binding properties, which would be important to their integration into the crystalline matrix.

More recently [120] four proteins from the organic matrix of CaOx crystals present in the seeds of Phaseolus vulgaris, have been isolated which inhibited the nucleation of CaOx crystallization in solutions. They have also shown that the isolated proteins modified the morphology of CaOx crystal mainly at {120} face (fastest growing face).

1.11. Commonly used phytotherapy for urolithiasis and their mechanism of action

Throughout human history people have used phytotherapeutic remedies to cure illness and improve quality of life. Various plant extracts exert their antilithiatic properties by altering the ionic composition of urine, e.g. by decreasing the calcium ion concentration or increasing magnesium and citrate excretion in urine. These extracts may also express diuretic activity or they contain saponins that can disaggregate suspensions of mucoproteins, which are actually promoters of the crystallization process.

The table 1.5 lists currently consumed phytotherapeutic agents that have been evaluated by in vivo and in vitro studies. Of these, Herniaria hirsuta, one of the most widely used herbal remedies, is a dried powder of Caryophyllaceae that grows in Qujda City, Morocco [121]. Atmani et al reported that Herniaria hirsuta progressively decreased the adhesion of COM crystals to canine kidney cells and Herniaria hirsuta did not appear to adversely affect cell growth under the conditions in their study [122].
**Review of Literature**

**Table 1.5.** Currently consumed phytotherapeutic agents and their mechanisms of action

<table>
<thead>
<tr>
<th>References</th>
<th>Agent</th>
<th>Evaluated</th>
<th>Potential Beneficial Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmani [121]</td>
<td><em>Herniaria hirsuta</em></td>
<td>Urine or cell culture in vitro</td>
<td>Removes crystals already attached to cell surface, results in higher COD vs COM excretion</td>
</tr>
<tr>
<td>McHarg [124]</td>
<td><em>Cranberry juice</em></td>
<td>Humans in vivo</td>
<td>Increases urinary citrate excretion, decreases urinary oxalate and calcium ion excretion</td>
</tr>
<tr>
<td>Trinchieri [125]</td>
<td><em>Grapefruit juice</em></td>
<td>Humans in vivo</td>
<td>Increases urinary citrate excretion</td>
</tr>
<tr>
<td>Seltzer [126]</td>
<td><em>Lemonade juice</em></td>
<td>Humans in vivo</td>
<td>Increases urinary citrate excretion</td>
</tr>
<tr>
<td>Garimella [127]</td>
<td><em>Dolichos biflorus</em></td>
<td>Urine or cell culture in vivo</td>
<td>Decreases calcium phosphate precipitation</td>
</tr>
<tr>
<td>Garimella [127]</td>
<td><em>Bergenia ligulata</em></td>
<td>Urine or cell culture in vivo</td>
<td>Decreases calcium phosphate precipitation</td>
</tr>
<tr>
<td>Schwartz [128]</td>
<td><em>Vigna unguiculata</em></td>
<td>Humans in vivo</td>
<td>Increases urinary magnesium</td>
</tr>
<tr>
<td>Grases [129]</td>
<td><em>Zea mays</em></td>
<td>Animals in vivo</td>
<td>Diuretic</td>
</tr>
<tr>
<td>Khan [130]</td>
<td><em>Amni visnaga</em></td>
<td>Animals in vivo</td>
<td>Diuretic</td>
</tr>
<tr>
<td>Selvami [131]</td>
<td><em>Aerva lanaea</em></td>
<td>Animals in vivo</td>
<td>Decreases urinary calcium, oxalate, uric acid &amp; phosphorus excretion</td>
</tr>
<tr>
<td>Viel [1321]</td>
<td><em>Costus spiralis</em></td>
<td>Animals in vivo</td>
<td>Decreases stone size with unknown mechanism, no diuretic effect</td>
</tr>
</tbody>
</table>

41
Membrane fluidity correlates with crystal adhesion to cells and crystals bind to cells more avidly when fluidity increases. Although *Herniaria hirsuta* extract seems to exert a minimal decreasing effect on crystal adhesion *in vitro*, this effect is more significant when the temperature increases from 20°C to 40°C. In addition to the surface blocking effect of this plant extract, *Herniaria hirsuta* could remove crystals already attached to cell surface. On the other hand, the extract alters human urine crystallization and oxalate addition results in more but smaller crystals. These results can be considered negative because the extract increases the number of crystals. However, crystalluria is not the only risk factor in urinary stone disease because it can also be observed in normal individuals [122]. Crystal size seems to be more effective in stone disease because larger crystals carry a higher risk of retention in the urinary tract, which should be considered pathological in the process of stone formation. Thus, although the number of crystals increases in urine, nucleation and aggregation might decrease. Moreover, the herb extract resulted in higher COD excretion than COM excretion. This may be considered another antilithiatic effect of the extract because COD crystals bind less tightly to epithelial cells [123].

Fruit juices can be another effective model of urinary stone disease treatment. On the other hand, the effect of alkalizing beverages together with citrus fruit juice ingestion on the risk of CaOx, uric acid and cystine lithiasis is still debated. Cranberry juice is a popular herb and McHarg et al [124] has investigated the antilithiatic effect of this juice. In their study they found increased urinary citrate excretion together with decreased urinary excretion of oxalate and calcium ions *in vivo*. Decreased oxalate excretion is especially important because this ion is a key risk factor for CaOx stone formation [133]. On the other hand, Kessler et al [134] studied a combination of cranberry, black currant and plum juice. They found that cranberry juice decreased urinary pH with an increase in urinary oxalic acid. From these results they concluded that cranberry juice acidifies urine and it could be useful for treating brushite and struvite stones as well as for urinary tract infection. Grapefruit juice is another widely studied herb for urinary stone disease [125]. These studies show that this juice increased the urinary excretion of citrate and
magnesium together with mean oxalate and calcium levels. In addition, there was no change in supersaturation, crystal aggregation or growth inhibition. Seltzer et al studied the effect of lemonade to treat hypocitruric calcium nephrolithiasis [126]. They reported that treatment for hypocitruric calcium nephrolithiasis requires full patient compliance and cooperation due to the high number of tablets, liquid supplements and numerous crystal packages. They concluded that lemonade juice could be considered an alternative or adjunct treatment because this juice leads to a 2-fold increase in urinary citrate. In contrast, these studies were performed in small samples using different treatment doses. Also, results were interpreted at different times with different methods. From this point of view these juices should be evaluated in prospective, double blind, randomized studies in larger sample sizes to reach a final conclusion.

*Phyllanthus niruri* is a member of the Euphorbiaceae family with a worldwide distribution [135]. It is used in Brazilian folk medicine for urolithiasis [135]. *In vivo* studies revealed that this extract significantly decreased stone growth in rat models and no toxic effect was reported after ingesting *Phyllanthus niruri* tea during 3 months [136]. Aqueous extract of *Phyllanthus niruri* significantly decreased CaOx crystal endocytosis and, moreover, *Phyllanthus niruri* promoted the adsorption of glycosaminoglycans into the kidney stone, making them softer and smaller in a rat model [137]. In addition, Barros et al reported that in their model *Phyllanthus niruri* extract induced an increase in the COD fraction, which can be considered an antilithiatic effect because it has been suggested that COM has stronger affinity for cell membranes than COD [135]. Their results, which are consistent with another complementary study performed by Freitas et al [138] also revealed that *Phyllanthus niruri* did not affect CaOx nucleation, but rather inhibited crystal growth since the particles were significantly smaller than control samples. Another potential effect of some herbal remedies is claimed to arise from their diuretic activity. *Anni visnaga*, a popular Saudi folk medicine, is an example of this diuretic effect [130]. Khan et al attributed the antilithiatic effect of this drug to its diuretic activity in maintaining the oxalate concentration below the supersaturation level at which precipitation as CaOx crystallization occurs.
Recent years have seen dramatic advances in phytotherapy for urolithiasis. An unavoidable interest in this results in an expense of more than $1.5 billion annually in the United States [128]. Although phytotherapeutic extracts are popular in folk culture, to our knowledge there are no reports in the literature of the exact clinical role, efficacy and side effects of these herbs after long-term consumption. Correspondingly, potential acceptance of this herbal therapy as an alternative or an adjunct to classic medical therapy remains to be determined.

Although increased excretion of urinary citrate, decreased excretion of urinary calcium and oxalate, and diuretic and antiseptic features are only some of the known mechanisms of these extracts, a precise understanding of the mechanism of action of these extracts would have diagnostic value in regard to the nature of this disease, in addition to the potential therapeutic implications in this future field of research. In this respect, absence of this information is a fruitful area for scientific research by willing investigators. Although preclinical research has proved that the efficacy of some of these herbs is truly mythical, all deserve innovative scientific study to clarify the mechanism of action because myths may always become reality in the future.