CHAPTER 1
INTRODUCTION

1.1 Urolithiasis: Kidney stone

Urolithiasis is a global problem afflicting human beings for several centuries. It is also called Nephrolithiasis or kidney stones. Urolithiasis is the presence of uroliths/calculi (stones) in the urinary tract. The annual incidence of urolithiasis in the western world is 0.5%. Urolithiasis has been found in the tombs of Egyptian mummies at 4000 BC and in the graves of North America Indians from 1500-1000 BC. Reference to stone formation is made in the early Sanskrit documents in India between 3000 and 2000 BC. The first documented urinary tract stones were found among the 7000-year-old boy teenage mummy in El-Amara (Probert, 2008; Bahuguna et al., 2009). The yearly incidence of urolithiasis is estimated to be about 0.55 in North America and Europe. Urolithiasis is a recurrence disease with a relapse rate of 50% in 5-10 year and 75% in 20 year. It is estimated that 12% of world population experience renal stone disease with a recurrence rate of 70-80% in male and 47-60% in female (Orson, 2006; Soundararajan et al., 2006). The life time risk of developing urolithiasis is about 10-15% in the Western world, but can be as high as 20-25% in the Middle East (Coe et al., 2005). Annual incidences of kidney stones are about 0.1-0.4% of the population and lifetime prevalence in the United States and Europe range between 8-15% (Daudon et al., 1993; Hess, 2003). In India, 12% of the population is expected to have urinary stones, out of which 50% may end up with loss of kidneys and renal damage (Mohamed et al., 2006). Urinary stones have been found to contain phosphate, uric acid, magnesium ammonium phosphate with apatite and struvites. Among the urinary stones, calcium containing stones are the common comprising about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) and a mixture of both (45%). Urolithiasis is the formation of calculi, or condition associated with urinary calculi. The term calculi are synonymous with uroliths, stones, or crystals. These calculi are formed by deposits of polycrystalline aggregates composed of varied amount of
crystalloid and organic matrix. They can vary in size and may be found anywhere in the urinary tract from the kidney to the bladder. The presence of clinical signs depends on the obstruction of urine, the presence of infection and edema (swelling). Stones that cause obstruction to the flow of urine set up an environment of urine stasis and bacterial growth. The irritation caused by the stones result in secondary infections leading to pyelonephritis (inflammation of the kidney) an upper urinary tract infection, or cystitis (inflammation of the bladder) and urethritis (inflammation of the urethra) a lower urinary tract infection. Though the cause of stone formation is hard to determine, some factors include a genetic predisposition, metabolic disorders such as diabetes, myeloproliferative disease like leukemia, or hypocalcaemia (abnormally high amounts of blood calcium), diet imbalance, a poor intake of water and bacterial infections such as *Escherichia coli*, *Klebsiella*, *Staphylococcus*, or *Mycoplasma*. Stones are often more commonly found in males than females due to a longer urethra. In addition, once stone formation has been found to exist and if treatment is able to resolve the stones that are present, it is known that there is still a high rate of recurrence (Abraham *et al*., 1984).

### 1.2 Pathophysiology of urinary stone

The stone formation requires supersaturated urine. Super-saturation also depends on urinary pH, ionic strength, solute concentration and complexations. Three conditions must coexist for the formation of Struvite calculi.

1. Alkaline urine,
2. The presence of urea or ammonia in the urine and,
3. Higher concentration of minerals in the urine.

Urine from healthy humans consists of a large quantity of nitrogenous compounds, including 0.5 M urea, as well as inorganic ions (Malhota, 2008). Urine is neutral to slightly acidic, and under these conditions, ammonia becomes protonated with the concomitant generation of hydroxide, which increases urine pH. The alkaline pH causes the precipitation of normally soluble polyvalent cations and anions in urine, leading to the formation of urinary stones.
Kidney stones contain calcium, oxalate, phosphate, magnesium, uric acid and the formation of urinary calculi involves a crystallization process that includes

- Nucleation,
- Growth and
- Aggregation of crystals.

Stone formation may be either homogeneous (where the nucleus of the stone around which crystals aggregate is the same material as that of the crystal) or heterogeneous (Atmani, 2003b).

1.2.1 Nucleation

Nucleation is the formation of a solid crystal phase in a solution. It is an essential step in stone formation. The term supersaturation refers to a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances (Barros et al., 2003). The point at which saturation of a solution is reached, and crystallization begins is commonly known as thermodynamic solubility product. Urine contains inhibitors of crystallization and can hold large concentrations of solute above the metastable state (Malhota, 2008). If the concentration of solute increases further and a point is reached where it cannot be held in solution, which is the point of formation of product in urine. The process of nucleation in a pure solution is known as homogeneous nucleation (Finlayson, 1978; Pearle and Nakada, 2009). In secondary nucleation, new crystals deposits on pre-existing crystal surface of similar type. Secondary nucleation results in the mass production of crystals. Epitaxy is a process whereby material of one crystal type is precipitated upon the surface of another whose lattice dimensions are almost identical (Lonsdal, 1968). Epitaxy is clinically important in the formation of calcium oxalate stones. These two processes are closely related to heterogenous nucleation.

1.2.2 Growth

After nucleation, crystal growth is the next major step in stone formation. The driving force of crystallization is a reduction in the potential energy of the atoms or
molecules when they binding to each other (Pearle and Nakada, 2009). Crystal growth is determined by the molecular size and shape of the molecule, the physical properties of the material, supersaturation solution level, pH and defects that may form in the crystal structure. Crystal growth is one of the prerequisites for particle formation (Qiu et al., 2004).

1.2.3 Aggregation
In this process, crystals in solution stick together and form a large particle. Aggregation of particles in solution is determined by a balance of forces, some with aggregating effects and some with disaggregating effects. A small interparticle distance increases attractive force and favours particle aggregation. 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 crystal is known as ‘Zeta potential’. In various steps of stone formation, crystal aggregation is an important factor than nucleation and growth because aggregation occurs within seconds (Aggarwal et al., 2000).

1.3 Kidney Stone
Kidney stone (called renal calculi) is solid concretions (crystal aggregations) formed in the kidneys from dissolved urinary minerals. The term ‘nephrolithiasis’ and ‘urolithiasis’ refer to the condition of having calculi in the kidneys and urinary tract, respectively (Figure 1.1(a)). Bladder stones can form or pass into the urinary bladder (Figure 1.1(b)). Ureterolithiasis is the condition of having a calculus in the ureter (the tube connecting the kidneys and the bladder). Usually, kidney stones form when the urine becomes concentrated to a great extent. This results in minerals, along with other substances, to form into crystals, which occur in the inner surface of the kidneys. In time, these crystals have the tendency to combine and to form a hard, small mass, or a kidney stone. The crystals that become kidney stones have a propensity of forming when the urine has high concentration of particular substance such as uric acid, oxalate, calcium, and sometimes, cystine. Kidney stones can also
form, if the body has low level of magnesium and citrate, which help in preventing crystal formation. Crystals also form if the urine is too alkaline, or too acidic, or if it becomes too concentrated.

1.4 Types of kidney stones

The most common varieties of calculi examined in our laboratory, calcium oxalate monohydrate (COM) and hydroxyapatite (HAP), have the potential to take on prickly, tentacle-like forms, generating a painful experience for the patient. However, the majority of stones examined are smooth and ovular. Other common components of calculi include Calcium oxalate (pure), Calcium oxalate in combination with aspartate, uric acid (pure), Calcium oxalate in combination with uric acid, Calcium oxalate dihydrate in combination calcium phosphate, struvite and Magnesium ammonium phosphate (Barbasa et al., 2002), as displayed in (Figure 1.2).

**Calcium stones:** The most common type of calcium stones are calcium hydrogen phosphate dihydrate (Brushite crystals) and calcium oxalate. Calcium is a normal part of a healthy diet. Calcium that is not used by the bones and muscles goes to the kidneys. In most people, the kidneys flush out the extra calcium with the rest of the urine. For some people this calcium gets accumulated and aggregates with other waste products to form stones.

**Struvite stones:** These stones normally develop after an infection in the urinary system. These stones contain the mineral magnesium and the waste product ammonia.

**Uric Acid Stones:** These stones form when there is too much acid in the urine. Metabolic disorders that cause improper acidification of the urine mostly end in the formation of uric acid stones. This can be prevented by reducing the amount of meat in diet.

**Cystine stones:** Their occurrence is mostly rare. Cystine is one of the building blocks that make up muscles, nerves, and other parts of the body. Excessive accumulation of cystine due to improper dietary factors can result in cystinuria, which ultimately ends up in stone formation.
1.5 Causes of kidney stones

Kidney stones may occur due to metabolic conditions, such as renal tubular acidosis (RTA), Dent's disease and medullary sponge kidney. Improper dietary factors contribute much towards kidney stone formation. Renal tubular acidosis (RTA) is a medical condition that involves accumulation of acid in the body due to failure of the kidneys to appropriately acidify the urine. When blood is filtered by the kidney, the filtrate passes through the tubules of the nephron, allowing for exchange of salts, acid equivalents, and other solutes before it drains into the bladder as urine. The metabolic acidosis that results from RTA may be caused either by failure to recover sufficient (alkaline) bicarbonate ions from the filtrate in the early portion of the nephron (proximal tubule) or by insufficient secretion of (acid) hydrogen ions into latter portions of the nephron (distal tubule).

Dent's disease is a rare X-linked recessive inherited condition that affects the kidney. It is characterized by tubular proteinuria, hypercalciuria, calcium nephrolithiasis, nephrocalcinosis and chronic renal failure. "Dent's disease" is often used to describe an entire group of familial disorders, including X-linked recessive nephrolithiasis with renal failure, X-linked recessive hypophosphataemic rickets, and both Japanese and idiopathic low molecular weight proteinuria. Medullary sponge kidney is a congenital disorder of the kidneys characterized by a cystic dilatation of the collecting tubules in one or both kidneys (Moe and Orson, 2006). Cystinuria and hyperoxaluria are two other rare, inherited metabolic disorders that often cause kidney stones. In cystinuria, there is too much of the amino acid cystine, which does not dissolve in urine and it is voided. This can lead to the formation of stones made of cystine. In patients with hyperoxaluria, the body produces too much of the salt oxalate. When there is more oxalate than can be dissolved in the urine, the crystals settle out and form stones. It has been estimated to occur with a prevalence of between 1 in every 10,000 individuals in the population. In most cases, medullary sponge kidney affects only one kidney, in which case kidney function is usually preserved and individuals may be asymptomatic. However, these individuals are at increased risk for nephrolithiasis (kidney stones) and urinary tract infection.
1.6. Review of Literature

The treatment and removal of renal calculi is often dependent upon the composition and hardness of the stone, as well as stone size and placement in the urinary tract. Though the main components of renal stones tend to be calcium oxalate, calcium phosphate (hydroxylapatite), magnesium ammonium phosphate and uric acid were found (Otnes, 1983; Dietrich et al., 1990). Treatment options vary depending on the placement of the stone in the renal system. The most preferable and least invasive treatment is, of course, natural passing, though this option is often the most uncomfortable for the patient. The option of natural passing is dictated by the size of the renal stone, since blockage of the urethra will occur with renal stones larger than approximately 6 mm in diameter. Intractable renal stones are treated through several different techniques, two of the more well-known methods being Extracorporeal Shock Wave Lithotripsy (ESWL) and surgical removal. Unfortunately, ESWL causes renal tissue trauma and hemorrhaging with virtually every treatment (Lingeman, 1992), while surgical methods are invasive and require longer recovery times (Goldfarb and Coe, 1999).

Extracorporeal Shock Wave Lithotripsy (ESWL), commonly known as lithotripsy, is a technique that uses shock waves produced outside the body to hit and break up the stones so that they can pass out of the body. Percutaneous nephrolithotripsy (the shattering of stones) and percutaneous nephrolithotomy (the removal of stones) involves the insertion of a narrow endoscope through the back of the patient into the kidney in order to break or remove the stone, respectively. This procedure is often used when ESWL has failed and is usually attempted prior to open surgery, which is considered as the last resort. Ureteroscopy is similar to percutaneous nephrolithotomy, but in this case, the endoscope is inserted through the urethra instead of through the back of the patient (Orson, 2006).

There are recent concerns as to the long term side-effects of lithotripsy and the damage caused to the renal system. Current research indicates that in addition to soft tissue damage, the development of brushite renal stones is directly related to
treatment by ESWL, where HAP, COM, or UA stones are removed but brushite stones are formed in their place (Lingeman, 2003). In addition to the surgical management of the problem there are certain drugs and other conventional therapies which are recommended for its control. A few numbers of drugs (Pearle et al., 1999) and some dietary measure (Borghi et al., 2002) have been shown in randomized trials to reduce the rate of stone recurrence. Indeed, a meta-analysis of randomized medical therapy trials showed a 22.6% risk reduction in stone recurrence rates with the initiation of drug and dietary therapy.

Besides, these treatments cause undesirable side effects such as hemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and recurrence of renal stone formation (Terlecki et al., 2007). The kidney stone forming patients are prone to its recurrence even after its surgical removal. Recurrence rates are close to 50%, and the cost of urolithiasis to individuals and society is high (Sutherland et al., 1985). Urinary stone is characterized by high recurrence rate therefore requiring a preventive treatment by using medicinal plants or phytotherapy. From the above facts it is clear that there is a need to study herbal plants for the treatment of urinary stones. Moreover, it is also beneficial for the mankind for its efficacy, safety and quality.

Existence and survival of mankind is impossible without plant kingdom, as plants are the primary procedure and play vital role in sustaining the life from earth. Human beings has used higher plants as a source of drugs of combat diseases since ancient times. Our indigenous systems of medicine namely ayurveda, siddha and unnani have been in existence for several countries (Pullaiah, 2006: Satish et al., 2009). The traditional systems of medicines and the folklore of various countries depend on medicinal plants for their medicinal preparations (Edwin et al., 2005). According to World Health Organization (WHO) estimate, 80% of population living in developing countries almost exclusively uses herbal medicines (Gupta and Chadha, 1995; Pareek, 1996). This means, that 3330 million people use medicinal plants on regular basis (Dahanukar et al., 2000). Council for Scientific and Industrial Research
(CSIR), New Delhi, is already involved in this field and validated about 350 formulations for different activities. This is a welcome trend, since it attempts to many traditional practice with modern knowledge for the betterment of health (Gupta and Chitme, 2000). WHO has emphasized the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Several countries have herbal pharmacopoeias and lay down monographs to maintain their quality. Ayurvedic Pharmacopoeia of India recommends basic quality parameters for 80 common herbal drugs (Dobriyal and Narayana, 1998). Plants and other natural substances have been used as the rich source of medicine. Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety, lesser side effects and better compatibility with human body.

However, few investigators have devoted their efforts to study their medicinal plants by using scientific methods. Such studies are needed to understand the mechanism by which these herbal plants exert their effects and identify their action principles. In this regard, herbal plants presently available for the treatment of urinary stone are as follows:

Didymocarpus pedicellata, commonly known as Patharphodi or Shila pushp is useful for stones of kidney and bladder. Rhabdia lycioides, also known as Pashanbhed is useful for stones in bladder (Shrivastava, 1971). In Susruta Samhita “Kurantika” or “Sitivaraka” (Celosia argental) is tested in ‘Viratarvadigana’, which have specific action in urinary diseases viz. calculi (ashmari), gravels (sarkara), dysuria (mutra krichhra) and suppression of urine etc. Celosia argental is considered to be specific for the treatment of ashmari that is urinary stone (Shah et al., 1972). Ashmarighana (destroyer of stone) by charak and Sushruta. Sushruta efficacy in vataj Ashmari with the characteristics of oxalate stone. Clinical investigations have also shown that spontaneous passage of stones within 8-10 days of treatment with Dolichos biflorus (Singh and Kumar, 1973). The aqueous extract of Bergenia ligulata produced maximum inhibition of calcium phosphate and calcium oxalate crystals growth under in vitro conditions (Seth et al., 1974). In unani system of medicine Tribulus terrestris
and while in homoeopathic system of medicine Berberis vulgaris, Cantharis spp., and Lycopodium spp. are being used for the treatment of diuretic and kidney stones (Kapoor and Kapoor, 1976). The roots of Rubia tinctorum was effective against calcium oxalate, calcium phosphate, calcium carbonate and uric acid type of urinary stones and also expel the stones by stimulating the smooth muscles of the urinary bladder (Schneider et al., 1979). Successful treatment of urinary tract infection and urinary calculi with Cystone (Ghose et al., 1980). Ureteric calculus disappeared within 55 days of treatment with Cystone a herbomineral composition (Muthusamy and Muthu, 1980).

A combined extracts of Crataeva nurvala, Tribulus terrestris and Dolichos biflorus has found to be effective in preventing the deposition of some material on glass beads in the urinary bladder of rats (Kumar et al., 1981). Oral administration of indigenous herbomineral drug Calcury (2 tds) in 40 cases of ureteric calculi, showing passing of disintegrated or intact stones through urine in 25 cases (Yadav et al., 1981). Aqueous decoction of Celosia argental is used for the dissolution and excretion of stones (Dubey et al., 1982). Cystone has also been found to be useful in urolithiasis, crystalluria and urinary tract infection in human beings (Chatterjee, 1982). Today, many herbal formulations are commercially available which are used for kidney stone management. The marketed composite herbal formulations, Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India), Uriflush (Inti Sumatera Global, Indonesia), Uriflow (Discovery Herbs, USA) and Chandraprabha bati (Baidyanath, India) have been widely used clinically to dissolve urinary stones in the kidney and urinary bladder (Jethi et al., 1983). In India, in the Ayurvedic system of medicine Pashanabhedha is the Sanskrit term used for a group of plants (Bergenia ligulata, Alternanthera sessalis, Aerva spp., Rotula aquatic, Ammaunia baceifera) with diuretic and antiurolithiatic activities (Mukherjee et al., 1984). Rice bran therapy was evaluated in patients with idiopathic hypercalciuria and it was noticed a reduction of urinary calcium excretion (Ohkawa et al., 1984). Numerous studies have demonstrated that magnesium is a potent inhibitor of calcium phosphate and calcium oxalate crystallization inhibiting especially crystal nucleation.
and growth under in vitro (Li and Blacklock, 1985; Kohri et al., 1988). In another study conducted in 164 hypercalciuric patients with calcium containing urinary stones, the frequency of stone episodes was reduced from 0.462 to 0.101 per patient per year (Ebisuno et al., 1986). Cystone is a product of the Himalaya Drug Co. which is often prescribed by the physicians to the patients suffering from urinary calculi. There are various studies which showed its ability to inhibit calcium phosphate and calcium oxalate mineralization (Sengupta, 1987). Pharmacological and clinical studies carried out on a composite herbal formulation, Trinapanchamool consisting of five herbal drugs namely Desmostachya bipinnata, Saccharum officinarum, Saccharum nunja, Saccharum spontaneum and Imperata cylindrica found it to be effective both as a prophylactic in preventing the formation and as a curative in dissolving the pre-formed stones in albino rats. The antiurolithiatic activity of this formulation has been attributed to its diuretic activity (Singh and Sachan, 1989). 

*Mimosa pudica* being to Fabaceae family was not effective in either preventing stone deposition or dissolving performed stones (Joyamma et al., 1990).

The ethanol extracts of *Crataeva nurvala* has an antiurolithiatic activity induced by foreign body insertion method using glass beads in albino rats (Singh and Usha Kapoor, 1991). The fresh juice of *Coleus aromaticus* was found to reduce the deposition of calcium and oxalate in the kidney of experimental rats (Bhaskar et al., 1992). *Rosa canina* was found to be effective on calcium oxalate urinary stone as it decreased calciurea and increased citraturia (Grases et al., 1992). Studies on the stem juice of *Musa paradisiaca* were found to be effective in dissolving the phosphate type of stones in albino rats induced by foreign body insertion method using zinc discs (Kailash et al., 1992). *Zea mays* has found to be diuretic activity and decoction of *Zea mays* obtained from female inflorescence or immature cobs are given twice daily for 7 days to expel stones from kidney (Grases et al., 1993). In another experimental study stem juice of Musa significantly reduced the incidence of oxalate urolithiasis by lowering the activity of the enzyme glycolic acid oxidase (Prasad et al., 1993). The stem juice of Musa reduced urinary oxalate, glycolic acid, glyoxylic acid and phosphorus excretion in hyperoxaluric rats (Poonguzhali and Chegu, 1994). Ethanolic
extract of *Ammania baccifera* significantly reduced phosphate type of urinary stones (Prasad *et al.*, 1994). The aqueous methanolic fraction of *Tribulus terrestris* is more effective against experimentally induced urolithiasis by foreign body insertion methods using glass beads in albino rats (Anand *et al.*, 1994a). The aqueous extract of *Tribulus terrestris* showed antiurolithiatic activity on the oxalate metabolism in male rats fed with sodium glycolate, and also revealed decrease in urinary oxalate excretion and in liver GAO and GAD activities and significant increase in urinary glyoxylate excretion (Sangeeta *et al.*, 1994). After consumption of tea prepared from *Hibiscus sabdariffa* increased uric acid excretion and also decreased uric acid after consumption of juices prepared from *Hibiscus sabdariffa* (Kirdpon *et al.*, 1994).

*Andrographis paniculata* belongs to Acanthaceae is beneficial for the treatment of post-ESWL urinary tract infection (Muangman *et al.*, 1995). Oral administration of *Herniaria hirsute* reduced the deposition of crystals in the kidney in albino rats induced by calcium oxalate nephrolithiasis (Grases *et al.*, 1995). The ethanol extract of *Homonia riparia* was found to be effective in reducing deposition of calcium in the kidney of both prophylactic and curative group animals A combined extract of *Aerva lanata, Ammania baccifera, Mimosa pudica, Rotula aquatica* had inhibitory effect on struvite and calcium oxalate stones (Prasad *et al.*, 1997). In addition, its efficacy to reduce urolithiasis was also reported in male Wister rats. In various reports, the anticalcifying properties of Cystone are used as a reference for evaluating the antilithiatic properties of other plants. Herbal therapy can reduce the recurrence rate (Mitra *et al.*, 1998). Aqueous extract of *Costus spiralis* when used reduced the growth of calcium oxalate calculi in the urinary bladder of rats (Viel *et al.*, 1999). Oral administration of *Alisma Orientale* (Takusha) in albino rats receiving ethyl glycol to induce CaOx stone formation prevented urinary stone by inhibiting CaOx aggregation (Yasui *et al.*, 1999).

Lupeol, a triterpene compound has been isolated from *Crataeva nurvala* showed antioxaluric and anticalciuric effects in rats against hydroxyproline-induced hyperoxaluria (Vidya and Varalakshmi, 2000). The two compounds viz., 7-hydroxy-2',4',5'-trimethoxyisoflavone and 7-hydroxy-4'-methoxy isoflavone isolated from the
heart wood of *Eysenhardtia polystachya* produced a significant decrease in urinary stone size was observed in animals treated with these compounds (Perez *et al*., 2000). Aqueous extracts *Rosmarinus officinalis* and *Centaurium erythraea* are used for kidney ailments (Louedec *et al*., 2000).

The extracts prepared from the seeds of *Dolichos biflorus* could inhibit calcium and phosphate type of crystals *in vitro*. A combined extracts of seeds of *Dolichos biflorus* and rhizomes of *Bergenia ligulata* were tested for their antilithiatic and anticalcification activity by homogenous precipitation method *in vitro* (Garimella *et al*., 2001). The seeds of *Ammi visnaga* showed highly potent diuretic activity and amelioration of uraemia and hyper bilirubinimia (Khan *et al*., 2001). Whole plant decoction of Chaya, along with castor (*Ricinus communis Linn.*) root and Gokhuru (*Tribulus terrestris Linn.*) fruits is given twice a day for two weeks to cure stones (Selvam *et al*., 2001). After consumption of a tea prepared from *Orthosiphon grandiflorus* increased of uric acid excretion and also stone size reduction in patients by increased excretion of calcium and uric acid (Premgamone *et al*., 2001). The chronic diuretic effects of the aqueous extract of the whole plant of *Spergularia purpurea* (SP) are used for kidney stone (Jouad *et al*., 2001).

The aqueous extract of root of *Randia echinocarpa* has an antiurolithiatic activity on albino rats (Vargas Solis *et al*., 2002). The root of *Cyclea peltata* has an inhibitory effect on stone formation and decreased urinary oxalate, calcium, serum potassium likewise increased serum magnesium levels (Christiana *et al*., 2002a). The ethyl acetate extract of *Rotula aquatica* has an antilithic activity against calcium oxalate and struvite type of urinary stones (Christiana *et al*., 2002b). The aqueous extract of *Phyllanthus niruri* has an inhibitory effect on calcium oxalate crystallization process by reducing calcium oxalate growth and aggregation *in vitro* (Freitas *et al*., 2002).

The aqueous extract had an inhibitory potential on calcium oxalate renal calculi induced by calcium oxalate seed in bladder of albino rats. The inhibitory effect due to higher levels of glycosoaminoglycans incorporated into calculi (Barros *et al*.,
The effects of *Vaccinium macrocarpon* (Cranberry) juice on urinary stone risk factors results due to the variability in the amount of juice ingested and causing decreased excretion of oxalate and phosphate (Mc Harg *et al.*, 2003). Oral administration of *Herniaria hirsute* reduced the deposition of crystals in the kidney in albino rats induced by calcium oxalate nephrolithiasis and also used for the treatment of lithiasis and block crystal binding to cultured renal cells (Atmani *et al.*, 2004).

The acute diuretic effects of the aqueous extract of the aerial parts of *Retama raetam* (RR) are used for kidney ailments (Maghrani *et al.*, 2005). The fresh juice of leaves of *Plectranthus amboinicus* reduced calcium oxalate renal calculi induced by administration of 1% ethylene glycolated water (Alvin Jose *et al.*, 2005). The ethanolic extract of *Asparagus racemosus* Willd. is more effective on lithiasis induced by 0.75% ethylene glycolated water to adult male albino wistar rats and also reduced the elevated level of calculogenic ions in urine and urinary concentration of magnesium (Christina *et al.*, 2005). The aqueous extract of *Bergenia ligulata* was studied in the presence of Artificial Reference Urine (ARU) and Human Urine (HU) and induced the growth behavior of calcium phosphate crystals under these conditions. The alcoholic extract of *Bergenia ligulata* is useful in dissolving the calculi developed by foreign body insertion method using zinc disc in the bladder of albino rats against phosphate type of urolithiasis (Vimal *et al.*, 2005a; Vimal *et al.*, 2005b). *Tamarindus indica* belongs to Fabaceae is widely used in Indian cooking and it contains high amount of tartaric acid. The effect of tartaric acid and tamarind solution has been found to be effective against calcium phosphate type of urinary stones (Joseph *et al.*, 2005).

Aqueous extract of *Melia azedarach* was studied against ethylene-glycol induced nephrolithiasis in male albino wistar rats and also reduced urinary calcium, oxalate, phosphate and elevated urinary magnesium levels and urine volume (Christina *et al.*, 2006). The aqueous and alcoholic extract of root wood of *Moringa oleifera* significantly reduced urinary oxalate synthesis in hyperoxaluria induced with ethylene glycol (Karadi *et al.*, 2006).
The extract prepared from *Quercus salicina* could suppress cell injury induced by oxalate exposure by scavenging free radicals and suppressing the activation of NADPH oxidates (Moriyama *et al.*, 2007). The Ethanolic extract of *Nigella sativa* L. seeds on ethylene glycol-induced kidney calculi in rats (Hadjzadeh *et al.*, 2007). *Citrus limon* (lemon juice) and *Citrus aurantium* (orange juice) are used as a natural substances to inhibit calcium oxalate crystallization under *in vitro* (Kulaksizoglu *et al.*, 2007). The aqueous extract of *Raphanus sativus* showed antilithiatic activity on calcium oxalate crystals induced by zinc disc in urinary bladder of rats (Vargas *et al.*, 2007). The aqueous extracts of *Tetraclinis articulate* produced maximum inhibition of calcium oxalate type of urinary stone *in vitro* (Beghalia *et al.*, 2007). The leaves of *Melia azedarach* have been selected for phytochemical investigation and antiurolithiastic activity on experimentally induced urolithiasis in rats (Yogendr and Bahuguna, 2007).

The juice of *Citrus medica* Linn dissolved struvite type of calculi to some extent in an *in vitro* model (Chauhan *et al.*, 2008a). The aqueous extracts of *Commiphora wightii* dissolved struvite type of calculi to some extent in an *in vitro* model (Chauhan *et al.*, 2008b). *Punica granatum* L. (Pomegranate juice) has an antiurolithiastic activity. After consumption of juice decreased urinary oxalate excretion and calcium oxalate deposition form in rats due to its antioxatative activity (Tugcu *et al.*, 2008). The aqueous extracts of *Semen plantagins* and *Folium pyrosiae* some of the traditional Chinese drugs are used to treat urinary stone by inhibiting the formation of calcium oxalate, the major component of the urinary stone (Yongtai *et al.*, 2008). The leaf juice of *Sesbania grandiflora* showed significant antiurolithiastic activity against calcium oxalate-type stones and also exhibited antioxidant properties (Doddola *et al.*, 2008). The acid fraction of extract of *Tamarix gallica* has an antiurolithiastic activity on calcium oxalate crystallization due to the presence of function of acid (Ahmed Bensatal and Ouahrani, 2008).

*Boerhaavia diffusa* dissolved struvite type of calculi to some extent in an *in vitro* model (Chauhan *et al.*, 2009). The aqueous and alcohol extracts of flowers of
Jasminum auriculatum are reported for kidney stone (Yogendr et al., 2009). Musa balbisiana Colla (Kela) belong to Musaceae family. Decoction of Musa roots and gulli (axis of maize cob, Zea mays Linn.) is given twice daily for 7 days in complaints of kidney and urinary tract stones and severe pain. Bryophyllum pinnatum (Lamk.) Oken. also called as Patharchata, Ajuba, Ghavpatta, Parnbeej belong to Crassulaceae. The fresh juice along with 2-3 kalimirch (Piper nigrum Linn.) powder is taken twice a day to expel stones. Equisetum debile Roxb. (Jode tode ki ghas) belong to Equisetaceae. Whole plant juice is given along with 1 gm Piper nigrum Linn. twice a day for 7 days for removal of both urinary tract and kidney stone (Prachi et al., 2009).

Terminalia arjuna bark has the potential to inhibit the formation of both calcium phosphate and calcium oxalate crystals in vitro. Butanol fraction of Terminalia arjuna extract was the most effective in inhibiting formation of calcium phosphate and calcium oxalate crystals in vitro (Chaudhary et al., 2010). Ethanolic extracts of Phyla nodiflora exhibited significant effect in preventing calcium oxalate stone formation and also dissolved the pre-formed calcium oxalate stones in the kidney along with sufficient effect on both in vitro and in vivo (Dodoala et al., 2010). The aqueous and alcohol extract of seeds of Macrotyloma uniflorum significantly reduced the elevated urinary oxalate showing a regulatory action on endogenous oxalate synthesis. The seeds of Macrotyloma uniflorum are endowed with significant antiurolithiatic activity and it also indicate that the alcoholic extract of Macrotyloma uniflorum shows better anti urolithiatic activity than aqueous extract (Anantha Krishna Chaitanya et al., 2010). The aqueous and ethanolic extract of seeds of Helianthus annuus Linn significantly reduced the elevated level of calcium oxalate ions which is considered as one of the inhibitor of crystallization (Khan et al., 2010). Pinus eldarica fruit extract prevents calcium oxalate deposition without producing dieresis (Hosseinzadeh et al., 2010). The aqueous extract of Hygrophila spinosa significantly reduced the elevated levels of these ions and protein in urine and also urinary concentration of magnesium. The elevated serum creatinne levels were reduced by prophylactic and curative regimen of extract treatment (Sathish et al., 2010).
Cynodon dactylon extract was able to reduce the growth of urinary stones in the rats and urine oxalate level was decreased in nephrolithiatic rats (Khajavi rad et al., 2011). The administration of ethanolic extract of Dichrostachys cinerea L to urolithiatic rats reduced and prevented the growth of urinary stones and reduced the elevated urinary oxalate and showing a regulatory action on endogenous oxalate synthesis (Jayakumari et al., 2011). Administration of Bergenia ciliate extract/cystone along with ethylene glycol showed significant protective effect in body weight and organ weight with few stray areas of calcifications in glomeruli. Bergenia ciliate extract shows higher renoprotective index than cystone (Sarmistha saha and Ramtej Jayaram Verma, 2011). Ethanol extract of dried roots of Musa paradisica Linn has a protective activity against renal calculi. Histology showed that large deposite of calcium oxalate crystals in all parts of the kidney (Jha et al., 2011). The possible effect of Coleus barbatus and Trigonella foenum graecum root extract and their combinations have possible effect on calcium oxalate urolithiasis in albino rats (Javed Khan Pathan et al., 2011). Supplementation with aqueous and alcoholic extracts of Aerva lanata, Dolichos biflorus and Musa significantly reduced the elevated urinary oxalate and also the deposition of stone forming constituents in the kidney of calculogenic rats were significantly lowered by curative and preventive treatment using extracts in rats (Ramachandran et al., 2011). Aqueous extract of Terminalia chebula, Glycyrrhiza globra, Nelumbo nucifera, Zingiber officinale, Hemidesmus indicus, Myristica fragrans and Citrus aurantofolia (Polyherbal formulation) has preventive effect in the formation of renal calculi, weight gain, decreased calcium and oxalate excretion, increased levels of urinary volume and stone inhibitors (Akila et al., 2011). Ethanolic extract of Bergenia ligulata and Nigella sativa and their combination can be assigned to be positive effect on the calcium oxalate urolithiasis in albino rats (Harsoliya et al., 2011). Supplementation with ethyl acetate extract of Ichnocarpus frutescens extracts significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The deposition of stone forming constituents in the kidney of calculogenic rats was also significantly lowered (Anbu et al., 2011).
Supplementation with ethanolic extract of *Kigelia pinnata* fruit significantly reduced the elevated urinary oxalate, uric acid and phosphate and decreased the deposition of stone forming constituents in the kidney of calculogenic rats (Ravindra et al., 2012). Methanol extracts of leaves of *Hyptis suaveolens* (L.) Poit showed comparable activity to that of cystone in terms of inhibiting the formation of calcium oxalate precipitate (Agarwal et al., 2012a). Polysaccharide from the brown seaweed *Sargassum graminifolium* (Turn.) ability to inhibit on calcium oxalate crystallization and its antioxidant properities DPPH (1,1-diphenyl-2-picrylhydrazyl free radical (Chao-Yan Zhang et al., 2012). Herbs and herbal drugs kanghi (*Abutilon indicum*), chaya (*Aerva lanta*), bishkapa or purnava (*Boerhaavia diffusa*), ajuba (*Bryo-phyllum pinnatum*), gokhuru (*Tribulus terrestris*), makka (*Zea mays*) etc have clinically proven effects like immune modulation, adaptogenic and antimutagenic, and they play a vital role in treatment of kidney stone disease (Tiwari et al., 2012). Administration of aqueous extract of *Achyranthes aspera* Linn roots to rats prevented urolithiasis induced with ethylene glycol and reduced the growth of calcium oxalate stones. The extract was also effective in reducing the renal tissue injury, decreasing the crystal size and facilitating easy expulsion and restoring normal kidney architecture (Aggarwal et al., 2012b). This study revealed information on 79 ethnomedicinal plant species belonging to 42 families being used for stone problem (Agarwal and Varma, 2012c). Crude methanol extract of *Plantago major* contained the active compound terpenoid, zyloric and potassium citrate significantly inhibited the area of crystal formation (Sharifa et al., 2012). Ethanolic fruit extract of *Cucumis trigonus* Roxb. is the most effective drug in inhibiting stone formation and healing renal damage caused by oxalate toxicity, thus confirming its antiurolithiatic property (Balakrishnan and Kokilavani, 2012). Hydroalcoholic extract of leaves of *Ceropegia bulbosa* restores oxalate, calcium and phosphorus levels to normal thus reducing the risk of stone formation (Mohd Azaz Khan and Debasish Pradhan, 2012). This review provides an information on urolithiasis complications, treatment with various herbal extract and some of the particular phytoconstituents responsible for antiurolithiatic activity (Satish et al., 2012). Aqueous *Costus igneus* extract promote the formation of
calcium oxalate dihydrate crystals and may possibly treat urinary stones by inhibiting the formation of calcium oxalate monohydrate crystals (Manjula et al., 2012a). Aqueous and ethanolic extract of Costus igneus (stem) and isolated compound lupeol and stigmasterol had an inhibitory effect on calcium oxalate crystallization in albino rats (Manjula et al., 2012b). Aqueous extract of stem and rhizome of Costus igneus can promote the formation of Calcium oxalate dihydrate (COD) crystals and reduce the nucleation rate of COM crystals, a major component of calcium urinary stone (Manjula et al., 2012c).

As evident from the above review, Herbal medicines play significant amount of benefits for the treatment of urinary stones because of efficacy, safety, lesser side effects and better compatibility with human body. The undesirable effect of the modern medicine has already diverted the attention of the people towards herbal medicines. To increase the acceptability and awareness among the people, there is an urgent need to develop trust and faith towards the safer indigenous system by establishing its validity in treatment for stone diseases. Health care systems are going to become more and more expensive, therefore we have to introduce herbal medicine systems in our health care. Let us hope that in future natural products will be competing modern medicines with added advantages of more safety and lower costs.

1.7 MEDICINAL PLANTS USED IN THIS STUDY

1.7.1 Tribulus terrestris

Taxanomical Classification

Kingdom : Plantae
Division : Mangnoliophyta
Class : Mangnoliopsida
Order : Zygophyllales
Family : Zygophyllaceae
Genus : Tribulus
Species : terristris
**Description**

Pubescent, procumbent annual 10-60 cm. stems simple or freely branched. Leaves opposite, often unequal paripinnate; pinnae 5-8 pairs elliptical oblong lanceolate. Flowers 4-5 mm, petals yellow, fruit of 5 stellately arranged, hard, rugose carpells which are keeled and tuberculate on the back, and with 2 or more stout spines on the sides (Figure 1.3(a)).

**Phytochemical constituents**

The occurrence of saponins, flavonoids, alkaloids, lignanamides and cinamic acid amides has been reported in *Tribulus terrestris*. Furostanol and spirostanol saponins of tigogenin, neotigogenin, gitogenin, neogitogenin, hecogenin, neohecogenin, diosgenin, chlorogenin, ruscogenin and sarsapogenin types are frequently found in this plant. The presence of spirostanol and furostanol saponins is a characteristic feature of this plant, the latter being considered to be biogenetic precursors of their spiro analogs (Anand *et al*., 1994a; Sangeeta *et al*., 1994; Vimal *et al*., 2005a; Vimal *et al*., 2005b).

1.7.2 *Tamarindus indica*

**Taxonomical Classification**

- **Kingdom**: Plantae
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Order**: Fabales
- **Family**: Fabaceae
- **Genus**: *Tamarindus*
- **Species**: *indica*

**Description**

Tamarind is a large tropical tree with the short massive trunk, ferny pinnate leaves, small yellow flowers and fat reddish pods. The tree can get 90ft tall but is usually less than 50ft. the leaves are about 10 inch long with 10 -18 pairs of 1in
oblong leaflets. Flowers are 1 in across, pale yellow with purple are red veins. The pulp that surrounds 8-10 seeds is both sweet and extremely sour (Figure 1.3(b)).

**Phytochemical constituents**

Tannin, saponin, sesquiterpenes, alkaloids, and phlobatanins are the common phytochemical constituents of this plant. It also contains two triterpenes, lupanone and lupeol (Joseph *et al.*, 2005).

1.7.3 *Crataeva nurvala*

**Taxanomical Classification**

<table>
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<th>Plantae</th>
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<td><em>Crataeva</em></td>
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**Description**

*Crataeva* is a deciduous tree, much branched. Leafs are alternate, palmately compound, three foliated. Inflorescence is dense terminal corymbs, bisexual (Figure 1.3(c)).

**Phytochemical constituents**

The parts of *Crataeva nurvala* generally contain flavonoids, glucosinolates, and plant sterols, including lupeol, saponins and tannins (Kumar *et al.*, 1981; Singh and Usha Kapoor, 1991; Vidya and Varalakshmi, 2000).
1.7.4 *Costus igneus*

**Taxonomical classification**

- **Kingdom**: Plantae
- **Order**: Zingiberales
- **Family**: Costaceae
- **Genus**: Costus
- **Species**: igneus

**Description**

Costus is a landscaping plant. Height: 1-1.5 m. A hardly ginger with dark green foliage and red cones which produce bright pink colour. Monocots (Figure 1.3(d)).

**Phytochemical constituents**

The parts of *costus igneus* contain tannins, saponins, sesquiterpenes, alkaloids and phlobatannins (Devi and Urooj, 2010).

1.7.5 *Grewia tiliacfolia*

**Taxonomical classification**

- **Cladus**: Eukaryato
- **Regnum**: Plantae
- **Order**: Malvales
- **Family**: Malvaceae
- **Subfamily**: Grewioideae
- **Genus**: Grewia
- **Species**: tiliacfolia

**Description**

Grewia is a deciduous plant, leaves are alternate, palmately compound, it has yellow flower (Figure 1.3(e)).
Phytochemical constituents

The parts of *Grewia tiliaefolia* contain a B-Carboline alkoloids, lupeol, betulin, tannin, saponin (Badami et al., 2003; Badami et al., 2004).

1.8 Animal model

Calcium oxalate kidney stones in both humans and mildly hyperoxaluria rats are located on renal papillary surfaces and consist of an organic matrix and crystals of calcium oxalate. Male Albino wistar rats were used in this study. Many in vivo models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agents on the development and progression of the disease (Fouad et al., 2004). Rats are the most frequently used animals in models of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans. Rat models of CaOx urolithiasis induced by either ethylene glycol alone or in combination with other drugs like ammonium chloride, are often used to study the pathogenesis of kidney crystal deposition (Khan and Glenton, 1995). In the present study rats were treated with 0.75% ethylene glycol for 28 days. All positive control rats (Group II) developed CaOx depositions during that time.

Urinary stone is characterized by its high recurrence rate if patients are not treated appropriately. The treatments used are Extracorporeal Shock Wave Lithotripsy (ESWL) and drug treatment. Even improved and beside the high cost that imposes, compelling data, now suggest that exposure to shock waves in therapeutic doses may cause renal injury, decrease in renal function and an increase in stone recurrence. In addition, persistent residual stone fragments and possibility of infection after ESWL represent a serious problem in the treatment of stones. Drug treatment has shown some feasibility in many randomized trials, it is not accomplished without side effect, which is sometimes very serious. Therefore, it is worthwhile to look for an alternative to these means by using medicinal plants or phytotherapy. So far the urinary stones are concerned, acupuncture, herbal medicine, natural products and homeopathy have been used to treat and/or to alleviate symptoms of kidney stone
patients. Herbal medicines play significant amount of benefits for the treatment of urinary stones because of efficacy, safety, lesser side effects and better compatibility with human body. This research provides a multidisciplinary approach in characterizing urinary stone CHPD and COM crystals grown in vitro and in vivo to help formulate prevention or dissolution strategies in controlling calcium urinary stone growth. This research is also focused to find new alternative medicine Costus igneus for the treatment of calcium oxalate urinary stone. Therefore, the purpose of this research is to investigate the beneficial effects of Costus igneus at a different dose and a single compound for the prevention of kidney stone formation.

Therefore, to test the hypothesis of this study the following objectives are proposed

1.9 Objective

- To investigate the composition and type of kidney stones in our population by FTIR spectroscopy.
- To grow urinary type CHPD (Calcium Hydrogen Phosphate Dihydrate), COM (Calcium Oxalate Monohydrate) and Struvite crystals in vitro and to evaluate the inhibition efficiency of aqueous extracts of fruits of Tribulus terrestris, leaves of Tamarindus indica, stem bark of Crataeva nurvala, stem of Costus igneus and stem of Grewia tiliaeefolia has been studied.
- The growth characteristics of the two most common types of kidney stones Calcium Hydrogen Phosphate Dihydrate (CHPD or Brushite crystals) and Calcium Oxalate Monohydrate (COM) and the effect of aqueous extracts of stem, leaf and rhizome of Costus igneus has been studied.
- The effects of aqueous extract of the stem of Costus igneus are used as additives to inhibit the nucleation and growth of CaOx crystals by both gel and liquid growth methods.
- The isolation, purification and characterization of one pentacyclic triterpenoid compound Lupeol and one steroid compound Stigmasterol from the stem of Costus igneus extract by HPTLC, HPLC, IR, $^1$H NMR, $^{13}$C NMR, TLC and Column chromatography technique separately.
➢ Assessment of inhibiting effect of aqueous and ethanolic extract of stem of *Costus igneus* and isolated compound using urolithiasis induced albino rats under *in vivo* condition.

➢ To examine the antibacterial activity of stem of *Costus igneus* and isolated constituents toward urinary tract infection causing pathogens using disc diffusion method.

1.10 Work Plan

To achieve the objective of the present study, the following work plan has been carried out.

**Chapter 2** Analysis of kidney stones by FTIR spectroscopy.

**Chapter 3** Growth characterization of Calcium Hydrogen Phosphate Dihydrate, Calcium Oxalate Monohydrate and Struvite crystals influenced by medicinal plants.

**Chapter 4** *In vitro* Evaluation of *Costus igneus* on Calcium phosphate and Calcium Oxalate Crystallization.

**Chapter 5** Growth characterization of Calcium Oxalate Monohydrate crystals influenced by *Costus igneus* aqueous stem extract.

**Chapter 6** Isolation, purification and characterization of Lupeol and Stigmasterol from the stems of *Costus igneus*.

**Chapter 7** Effect of *Costus igneus* stems extract on calcium oxalate urolithiasis in albino rats.

**Chapter 8** Antibacterial activity of the crude ethanolic extract and the isolated compounds from the stem of *Costus igneus*. 
Figure 1.1 (a) Bladder stone from human, (b) Kidney stone from human.
Figure 1.2 Types of Kidney stones from human samples (a) Pure calcium oxalate stone, (b) Calcium oxalate in combination with aspartate, (c) Calcium oxalate in combination with uric acid, (d) Uric acid, (e) Calcium oxalate dihydrate in combination with calcium phosphate and struvite (f) Magnesium ammonium phosphate.
Figure 1.3 Medicinal plants (a) Fruits of *Tribulus terrestris* (b) Leaves of *Tamarindus indica* (c) Stem bark of *Crataeva nurvala* (d) Stem of *Costus igneus* (e) Stem bark of *Grewia tiliaefolia*.