Chapter 2

REVIEW OF RELEVANT LITERATURE

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2. Introduction

The present study was undertaken to explore the possibilities of developing novel drugs for urolithiasis based on traditional knowledge of antiurolithiatric medicinal plants in Kerala coupled with advancement made in the area of phytochemistry and crystallography. A variety of indigenous remedies have been reported for the treatment of urinary stones. Hence, an attempt is made by the investigator to explore in detail the long-established botanical knowledge of traditional vaidyans and herbal collectors of Kottayam district with a view to identify the potential antiurolithiatric medicinal plants. The study also envisages to evaluate the antiurolithiatric property of selected medicinal plants by conducting and evaluating the in vitro crystallization and growth inhibition studies of Calcium Hydrogen Phosphate Dihydrate (CHPD) crystals. With this end in view, an abstract of relevant materials are presented here under appropriate headings.

2.1 Section A: Urolithiasis- An Overview

In this section an overview of urolithiasis with special reference to the history, relevance of Pashanabedha group of plants, prevalence, etiology, pathogenesis and medical management of urinary stones was attempted. Urolithiasis, kidney stones, renal stones, and renal calculi are interchangeably used to refer to the accretion of hard, solid, nonmetallic minerals anywhere in the urinary tract including the kidneys and the bladder. It is estimated that about 12% of the world population are afflicted by urinary stone disease. An in-depth analysis of the available literature revealed that no satisfactory drugs have so far been developed in modern medicine to disintegrate and dissolve the urinary stone and the patients by and large rely on alternative systems of medicine for their better relief.
2.1.1 History of Urolithiasis

Stone disease can be traced back to the earliest records in human civilizations. Archaeologic evidence and prehistoric documents revealed that stone formation had upset the mankind since the earliest known civilizations. The presence of urinary calculi had been found in the kidneys of several Egyptian mummies. The most ancient of these calculi was recovered from the pelvic remains of an Egyptian boy dated back from 4800 BC in a prehistoric tomb at el-Amrah near Abydos. It was found by G. Elliot Smith in 1901 and was presented to the Museum of the Royal College of Surgeons of England. The yellow stone weighed 12-g, with a uric acid nucleus and concentric laminations of calcium oxalate and magnesium ammonium phosphate (Shattock, 1905; Shah and Whitfield, 2002; Eknoyan, 2004).

Early literary (3200 - 1200 BC) references to stone disease were made within the medicinal texts called ‘Asutu’ in Mesopotomia. These medicinal texts present description of symptoms and treatments to dissolve the urinary calculi. They had the knowledge of soluble or insoluble (bladder) stones. For the dissolution of soft kidney and bladder stones, saltpeter and turpentine oil were used which increase the urine production, and pulverized egg-shell, mainly from ostrich eggs, with a high content of calcium carbonate, was ingested to bind lithogenic substances (Dardioti et al., 1997; Shah and Whitfield, 2002). The harder stones were left for surgical treatment. Hence, our typical current treatment strategies, and even invasive interventions such as urethral catheterization, were already ‘available’ centuries ago (Shah and Whitfield, 2002; Bitsori and Galanakis, 2004). Knowledge about old traditional Egyptian medicine for the treatment of diseases of the urinary tract, including stones, have already been found in the Papyrus Ebers which is the longest and the most famous source of our information concerning ancient medicines in Egypt. It was found in the tomb at Thebas at about 1862 and was acquired by the Egyptologist, Edwin Smith Papyrus. The Ebers Papyrus was written about 1500 BC (Cyril, 1930; Eknoyan, 2004). Urinary stone was well known to the ancient Greeks, and can be traced back to the period of Hippocrates. As it is evident from the Oath of Hippocrates (born 460 BC), he had profound knowledge about the clinical
symptoms of bladder stones. According to him, ‘The disease has five symptoms: pain when one wishes to urinate; passage of urine drop by drop as in strangury; blood-stained urine; the bladder being ulcerated by the stone; inflammation of the bladder and passage of the sand in the urine. Hippocrates adamantly stated that wounds of the bladder were lethal. To this date, lithotomy was practiced with only perineal incisions and the delay in suprapubic lithotomy may be ascribed to Hippocrates’ warning. In the Hippocratic oath he stipulates, ‘I will not cut persons labouring under the stone but will leave this to be done by practitioners of this work’. This admonition to physicians about a very risky procedure was to be held for centuries, but his reasons for this may never be known.

A lithotripsy procedure for the treatment of bladder stones was introduced by one of the most known Stein-Schneider of the Alexandrine epoch, who lived around 280 BC. However, such stone removal procedures were originally prohibited by the Hippocratic Oath, possibly due to the severe risks of the procedure (Sachs, 2003). Complications observed at this time were high fevers, urinary fistulas, impotence due to the perineal approach, and even death. Working men (surgeons), who were then not bound by the Hippocratic Oath, were hence the only persons able to perform such procedures.

Ammonius of Alexandria (276 BC) was the first person to suggest crushing the stone to facilitate its removal using a hook and a thin blunt ended instrument. Ammonius was given the nickname ‘lithotomus’, which was for the first time this word was used, referred to cutting the stone(Shah and Whitfield, 2002). It was later that this term is used for the operative procedure pertaining to stones.

Aulus Cornelius Celsus of the time 25 BC– 50 AD tried to include all medical knowledge into an encyclopedia. In this book, he reported on the typical (colicky) abdominal pain of patients with urolithiasis, but he also described the ‘Steinschnitt’ procedure with a perineal incision, which was called the Celsic method and was used until the late eighteenth century (Shah and Whitfield, 2002; Sachs, 2003). He recommended that the patients should take a long walk and a specific diet. He also reported on complications such as post-operative bleeds, fistulas and bladder tamponade.
The traditional Persian system of medicine date back to 1000 BC also describes the symptoms of stone disease and suggests avoidance of eggs, meat and fish. Other treatments include rose water, Indian beans, melon pips and a combination of substances where ‘one crushes melon and cucumber pips with radish, turnip and carrot seeds, and after the mixture have been boiled and filtered, the infusion was taken with a puree of radishes’. Within Arabic–Islamic medicinal literature, stone removal procedures were known and were frequently reported by Rhazes (865–925 AD) and Ibn Sina but especially from Abulcasis (936–1013 AD), who worked in Cordova, Spain, under the caliphate of the Omijades. In sixty chapters of his first book, ‘Surgery’, he reported on bladder and urethral stones, mostly in agreement with his predecessor Celsus (Abdel-Halim et al., 2003). He used clysters before lithotomy and discussed the complicated removal of huge bladder stones in detail. He seemed to be the first physician to crush stones within the urethra by means of an old version of the currently used lithotripter.

The treatment of bladder stones was popular in the sixteenth to eighteenth centuries, particularly because of the traveling of Stein Schneider. The prevalence of kidney stone disease had increase during this time and was found throughout all ages and social groups. The reason for this was the change in nutritional habits. After the pest epidemics, nutrition improved in the sixteenth century, with a steady increase in calorie intake (Shah and Whitfield, 2002).

During the late seventeenth century Frère Jacques Beaulieu (1651–1714 AD) together with Johann Jakob Rau invented the lateral approach for a perineal lithotomy (Eknoyan, 2004). He then performed approximately 5,000 lithotomies, but unfortunately his method was accompanied by severe morbidity and mortality (Ganem and Carson, 1999). As this stone removal procedure was very painful and combined with great mortality, it was only accepted as the ‘final way’ for the treatment.

At the end of the eighteenth century determination of stone composition, as well as urinary constituents, became possible. This led to the finding of excess excretion of uric acid in urine and thus ‘uric acid’ as a common stone component. Later, various other determinants of kidney stone disease were also unravelled;
with calcium and oxalate the more prevalent, the more rarely substances like xanthine or cystine were also found (Richet, 1995). In 1824, Jean Civiale first presented a lithotryptic instrument, which enabled him to crush and then remove a bladder stone via the urethra, at the Neckar Hospital in Paris (Dietrich, 2002). In 1826 he collected the data of patients from all over Europe to show the advantages of his method over the lithotomy procedure, expressing a one in five mortality rate for lithotomy and a significant lower mortality rate under the usage of the transurethral instrument. It should be noted that the latter method can only be used in patients with smaller stones that were within reach (Vandenbroucke, 2001).

2.1.1.1 History of Urolithiasis in India

The medical and surgical measures in the management of urological ailments prevailed in ancient India. Medical doctrines are first encountered in the religious texts called the Vedas compiled in successive generations from 3000 to 1000 BC (Mac Donell, 1962). The four Vedas namely the Rig, Yajur, Sam and Atharva Veda record the Vedic hymns as oral religious literature that are still recited during weddings, funerals and many socio-religious occasions in contemporary India.

The determining reference to urologic ailments in human history is encountered in the Atharva Veda dealing with urinary retention. It specifies the management with camphor and indigenous herbs to be anointed on the abdomen along with chanting of the appropriate hymns (Goswami, 2000).

During the Samhita period, the two stalwarts – Charaka in medicine and Susruta in surgery elevated the art of medicine in India to unprecedented heights. Their elaboration of the etiopathological hypothesis and the medical and surgical treatments of urolithiasis/urological disorders still remain valid to some extent in our contemporary understanding. The new generation of accomplished medical practitioners and researchers in urology should humbly venerate the practice of medical and surgical measures in the management of urological ailments prevailed in ancient India from the Vedic era around 3000 BC. The Charaka Samhita (Compendium of Caraka) is believed to be the oldest of the three surviving ancient treatises of Ayurveda. It is central to the modern-day practice of Ayurvedic
medicine and along with the Susruta Samhita (Compendium of Suśruta) was an important source of medical and life understanding and practice in antiquity (Valiathan, 2003). The main emphasis of Charaka was on the maintenance of a healthy disease-free ambience in life by achieving a balance of the three primary functional elements - Vayu (air), Pitta (bile) and Kafa (phlegm). Description of different types of stone disease (Ashmari) was given in Charaka Samhita. It contains several sections on urologic ailments (Nag, 1984). The entire fourth chapter of volume 2 is devoted to urinalysis and clinical interpretations based upon the color, consistency, turbidity, stickiness, presence of blood, semen, pus and fat in urine. Charaka analyzed the urinary findings with the symptoms of frequency, dysuria, polyuria, intermittency, fever, malaise, nausea etc to arrive at an etiopathological explanation of the individual ailments. Later in the same volume he discussed urinary retention precipitated by dietary and alcoholic indiscretions. In volume 5, chapter 26, Charaka mentioned the symptoms, frequency, strangury, hematuria and occasional urinary obstruction from vesical calculus. He also mentions the shape and surface characteristics of various calculi and offers his theories on the etiology.

Charaka also describes urethral and bladder instillations for certain calculus diseases and for cystitis in women. Various herbal medications are recommended for oral intake as well as to be anointed on the abdomen. In recalcitrant situations he advised referral for surgical intervention. Several chapters in Susruta Samhita deal with urinary tract infections. Susruta described various urological ailments with assumption about their pathogenesis followed by their detailed management. He also mentioned about a number of urethral probes, dilators and irrigating syringes for instillation of medications. Susruta ascribed the cause of calculi to four entities: phlegm, bile, air or semen, and gave the following description of their production; ‘When air and phlegm meet, a small stone is formed which grows towards the bladder outlet and hinders the outflow of urine. The suffering patients then grind his teeth, press his abdomen and rub his penis. Urine, flatus and faces are passed with severe pain. In such a case, the stone is black, rough, irregular and covered with spikes like the maneleacadamba flower’ (Bhishagratna, 1963). It is
assumed that the plant mentioned refers to *Neonauclea purpurea* (manjanircadambu) or *Neomarckia cadamba*, typical riverine tree species, belonging to the family Rubiaceae.

Susruta described several varieties of urinary calculi, their clinical manifestations and emphasized dietary indiscretion as the main etiological factor. In chapter 7 of Chikitsasthanam, Susruta mentions initial medical management with diet, fluids, alkali and bladder instillations. Surgery was suggested, only when these treatments failed. Susruta was one of the first to describe lithotomy in great detail. He listed pre-operative preparations to include anointing the body, cleansing of the system with emetics and purgatives, and prayers and offerings to the Gods. He proceeded to specify the position of the patient who ‘lies on his back, placing the upper part of the body in the attendant’s lap, with his waist resting on an elevated cloth cushion … knees and elbows contracted and bound’. The attendant is described as a person of ‘strong physique and unagitated mind’. The surgeon then rubs clarified butter into the left side of the umbilical region and ‘presses firmly with his fist downwards until the stone is as low as possible’. Sushruta’s description of the operation is interesting; ‘the left index and middle fingers, with cut nails, are dipped in oil and introduced into the rectum, and are pressed forward until the stone is grasped and stands out like a tumour’. Even at this stage, Susruta warns the surgeon that if the patient faints upon grasping the stone, the operation should be abandoned, otherwise ‘the patient will surely die’ (Desnos, 1972). The actual surgical procedure is also described in the text; ‘An incision is made on the left side of the raphe of the perineum at the distance of a barley corn and of sufficient width to allow the free egress of the stone. Several authorities recommend the opening to be on the right side of the raphe of the perineum for the convenience of the operation. It is also suggested to take special care in extracting the stone from its cavity so that it may not break into pieces nor leave any broken particles behind, however, small, as they would in such a case, be sure to grow larger in future (Hernam, 1915). Susruta proceeds to give postoperative instructions for the patient to sit in warm water, which was thought to prevent the accumulation of blood in the bladder. However, if blood does accumulate in the
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In the Ayurvedic system of medicine, ‘Pashanabheda’ group of plants were used for the treatment of urinary stones. ‘Pashanabheda’ is the Sanskrit term used for a collection of plants with diuretic and antiurolithiatic activities (Pashana = stone; Bheda = break). It is believed that these plants have the property of breaking and disintegrating the kidney stones. However, its identity is yet debatable. A large number of indigenous drugs have been used for this purpose in our country since ancient times. Susruta Samhita (170 AD- 340 BC) mentions the drug under various synonyms in Chikitsa silianam- under the name Pashanbhed for uric acid calculi and Ashnibhid for biliary calculi. In Susruta Samhita, decoction of Pashanbhed, Ashmantaka, Satavari, Vrihati, Bhalluka, Varuna (Crataeva nurvula), kulatha, kola and kataka seeds have been described for the patients of Vataja Ashmari, while Kusa, Ashmabhid, Patala, Trikantaka, Sirisha, Punarnava and Silajatu and Meduka flower for Pittaja Ashmari have been mentioned (Johnston, 1929). Ashtang Hridaya (341 AD-434 AD) mentions the drugs in Chiktsitsthnam- Upalbhed for extreme pain due to obstructed micturition, Pashanbhed for uric acid calculi and ashmabid for biliary calculi. In Susruta Samhita “Kurantika” or “Sitivaraka” (Celosia argental) is tested in ‘Viratarvadigana’, which is said to have specific action in
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urinary diseases, viz., calculi (ashmari), gravels (sarkara), dysuria (mutra krichhra) and suppression of urine.

Ashtang Hridaya (341 AD - 434 AD) mentions the drugs in chiktsitsthanam- Upalbhed for extreme pain due to obstructed micturition, Pashanbhed for uric acid calculi and ashmabid for biliary calculi. Aerva spp., Ammania baccifera and Nothosasrva brachiata have been reported from South India as lithotriptic plants (Mukhopadhyaya, 1929). Celosia argenta L. in Indian system of medicine is considered to be specific for the treatment of ashmari i.e., urinary stone. Aqueous decoction is used for the dissolution and excretion of stones (Bhishagratna, 1963). Didymocarpus pedicellata, commonly known as Patharphodi or Shila pushp is useful for stones of kidney and bladder, while Homonoia riparia, known as Pashanbhed or Kshudra Pashanbhed is useful in vesical calculi. Rotula aquatica syn. Rhabdia lycioides, also known as Pashanbhed is useful for stones in bladder. Bergenia ligulata, syn. Saxifraga ligulata, known as Pashanbheda have strong diuretic and lithotriptic activities but Kalanchoe pinnata syn. Bryophyllum calycinum known as Pashanbhed in Bengal, and others have no diuretic or lithotriptic activity. Bridelia Montana also known as Pashanbhed has also not shown any such activities (Mukhopadhyaya, 1913). Tribulus terrestris fruits have also been found useful in diuretic and kidney stones (Garrison, 1929). Effective cure of urinary calculi have been prescribed by practitioners in unani system of medicine (Castiglioni, 1947) while in Homoeopathic system of medicine, Berberis vulgaris and Lycopodium spp. are being used.

2.1.2 Prevalence of Urolithiasis

Prevalence of urolithiasis varies in different countries. In adults, the prevalence is relatively higher in Western countries than in the Eastern hemisphere. The reported prevalence of urolithiasis increases from 1-5% in Asia, to 5-9% in Europe, 12% in Canada and 13-15% in the USA, although even some Asian countries, such as Saudi Arabia, have a very high reported prevalence of 20.1% (Ramello, 2000; Lopez and Hoppe, 2010). In China, the overall prevalence rate was estimated to be 4.0%, 4.8% in men and 3.0% in women (Zeng and He, 2013). An extrapolated incidence statistics of 3,915,700 kidney stones has been estimated from
a population of 1,065,070,607 persons in India (http://www.census.gov). In Kerala too, owing to the strong association to the environmental aspects, growth incidence is expected to be highest as in regions that have dry and hot climatic conditions such as Asia. Global warming is also expected to significantly contribute to the increase in kidney stone incidence in the future (Trinchieri, 2006).

2.1.3 Etiology of stone formation

Several factors increase the risk for developing kidney stones, including inadequate fluid intake and dehydration, reduced urinary flow and volume, certain chemical levels in the urine that are too high (e.g. calcium, oxalate, uric acid) or too low (citrate), and several medical conditions (Kumar et al., 1991). Anything that blocks or reduces the flow of urine (e.g. urinary obstruction, genetic abnormality) also increases the risk of stone formation. The modern western lifestyle provides the factors that impair urine composition and thereby increasing the risk of stone formation. In our everyday life, we do not drink enough water, only twice or thrice a day, we eat food that is too rich in calories and table salt, but have deficiencies in fiber and alkali (Straub and Hautmann, 2005). The consequences are supersaturated urine and urine deficient in inhibitory substances which leads to the formation of urinary stones. Hyperoxaluria is a major risk factor of kidney stone disease (Straub and Hautmann, 2005). Oxalic acid present in foods such as chocolate, rhubarb or sweet potato and beverages is poorly absorbed from the intestine (Menon and Koul, 1992; Holmes and Kennedy, 2000). The exact etiology of increased urinary oxalate excretion remains to be elucidated. Increased dietary protein intake, altered renal excretion and increased hepatic oxalate production have all been postulated as possible causes. Nonetheless, the preponderance of evidence suggests that increased intestinal absorption is the primary cause for hyperoxaluria in patients with calcium oxalate stone disease (Menon and Koul, 1992). In short, the etiology of urinary calculus is complex and is considered multifactorial, such as food habit, altered urinary solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobilization, Randall’s plaque, microliths and urinary tract infection (Fowler, 1995). Renal, urologic, endocrine,
and metabolic disorders may also lead to the development of crystallized materials in the urinary system (www.emedicine.com/PED/topic 2371.htm).

2.1.3.1 The pathogenesis of renal stone

It is assumed that stone usually begins as tiny sand like speck of material (nidus) in the kidney. Minerals in the urine especially calcium, build on the speck in a similar way to that in which a pearl grows in an oyster shell. The formation of the nidus may be analagous to the first stage in the physiologic calcification of bone in which a nucleus of calcium phosphate develops in an organic matrix (Howard et al., 1967; Thomas and Howard, 1959; Boyce and King, 1963; Boyce, 1968). Alternatively, the nidus may form spontaneously by precipitation from supersaturated urine (Vermeulen and Lyon, 1968). It is reported that calcium phosphate is the solute in urine which crystallize soon and is reported to be present in small quantities in renal and urethral stones (Grases, 1993; Tiselius, 1996). Moreover, small amounts of calcium phosphate were detected in the assumed attachment part of the stone (Miller, 2007; Trinchieri, 2006; de Bruijn, 1995). These observations suggest that other stones have their origin on a calcium phosphate precipitate and that the early stages of these stones are attached to the renal papilla (Evan, 2009; Coe, 2010; Matlaga, 2006). Once the crystal nidus had established, it develops into a renal stone by precipitation or by the process of crystal growth. The high degree of internal structure usually found in the stone suggests an orderly addition of ions to the nidus by epistaxy (Neuman and Neuman, 1958) or under the influence of the inter-ionic forces of the nidus rather than by simple precipitation. A state of super saturation of urine in terms of calcium and phosphate ions is probably essential for the development of a renal stone of calcium phosphate composition, irrespective of the mechanism for the nidus formation and the growth of the stone (Mac Gregor et al., 1965). In short, kidney stone formation is a complex process that results from the succession of several physico-chemical events including supersaturation, nucleation, growth, aggregation, and retention within renal tubules (Khan, 1997).
2.1.4 Medical Management of Urinary stone

2.1.4.1 Treatment Options

The present medical management of urinary stone includes lithotripsy and surgical procedures which are prohibitively expensive for the common man and with these procedures recurrence is quite common and the patient has to be examined through careful follow up for several years (Christina, et al., 2002). Various factors such as size of calculi, severity of symptoms, and degree of obstruction, kidney function, location of the stone and the presence or absence of associated infection, influence the choice of one type of intervention over the other. Stones which are smaller than 5mm have a high probability of spontaneous passage which can take up to 40 days (Coll et al., 2002). During this watchful waiting period, patients can be treated with hydration and pain relieving medication. However, stones larger than 5mm or stones that fail to pass through urine are treated by interventional procedures (Knoll, 2007). Open surgical procedures for the treatment of ureteric stones have gradually disappeared in the last 30 years and have been replaced by minimal invasive techniques such as ESWL or ureteroscopy. ESWL is a noninvasive practice which uses shock waves to fragment calculi (Silberstein et al., 2008). This technique is the most extensively used method for managing renal and ureteral stones. However, treatment success rates depend on stone composition, size, properties and location of the stone as well as the instrumentation type and shock frequency (Knoll, 2007). It also needs to be considered that the same forces that are directed at the stones have deleterious effects on surrounding tissues. Damage to almost every abdominal organ systems have been reported but by far the most common injury is acute renal hemorrhage although its true incidence is unclear and poorly defined. Most often renal hemorrhage can be managed predictably; however, in rare instances the complications are fatal (Silberstein et al., 2008). Reports of post-ESWL perirenal hematoma range from less than 1% to greater than 30%. Besides, ESWL has been associated with long-term medical effects such as diabetes mellitus and hypertension (Krambeck et al., 2006).
In addition to ESWL, other procedures such as ureteroscopy (URS) have been developed for removal of ureteral stones. The new generations of ureteroscopes are flexible, smaller in diameter, stiffer and more durable, and have an improved tip deflection (Knoll, 2007). The major drawback of URS is that it is more persistent than ESWL and the rate of ureteric perforation and stricture formation remains around 2 to 4% (Pearle et al., 2001). In contrast, the major advantage of URS is that it is cheaper and results in higher and faster stone free rates (Nabi et al., 2007). It remains unclear which treatment modality is better than the other and the final decision is based on the patient’s preference, the size and the location of the stone, expertise of the physician and the costs of the procedure (Knoll, 2007).

2.1.4.2 Prevention Options

Regardless of the major technical attainments for stone removal in the last three decades the problem of recurrent stone formation remains. The recurrence rate of kidney stones is approximately 15% in the first year and as high as 50% within five years of the initial stone (Tiselius, 2000). Effective kidney stone prevention is dependent on the stone type and the detection of risk factors for stone formation. An individualized treatment plan incorporating dietary changes, supplements, and medications can be developed to assist in preventing the formation of new stones. Without considering the underlying etiology of the stone disease, patients should be instructed to increase their fluid intake in order to maintain a urine output of at least 2 L/d. A high fluid intake reduces urinary saturation of stone-forming calcium salts and dilutes promoters of CaOx crystallization. A high sodium intake boosts stone risk by reducing renal tubular calcium reabsorption and increasing urinary calcium. Patients should be advised to limit their dietary sodium intake to 2000–3000 mg/day (Park and Pearle, 2007). Restriction of animal proteins is also encouraged since animal proteins provide an increased acid load because of the high content of sulfur-containing amino acids. Thus, a high protein intake reduces urine pH and citrate and enhances urinary calcium excretion via bone resorption and reduces renal calcium reabsorption. Persons with a tendency of kidney stone formation should not be advised to restrict
calcium unless it has been shown that they have an excessive intake of calcium (Tiselius, 2000). A reduced intake of calcium leads to an increased intestinal absorption of oxalate, which itself may account for an increased risk of stone formation. Vitamin C has been implicated in stone formation because of in vivo conversion of ascorbic acid to oxalate. Therefore, a limitation of vitamin C supplementation to 500 mg/day or less is recommended (Park and Pearle, 2007).

When dietary modification is useless, pharmacological treatment should be initiated. The most effective hypocalciuric agents are thiazide diuretics which hypocalciuric action, enhance calcium reabsorption in the distal renal tubules (Laerum and Larsen, 1984). However, long term use (up to 50% of patients) is limited because of side effects including fatigue, dizziness, impotence, musculoskeletal symptoms, or gastrointestinal complaints (Park and Pearle, 2007). Another complication is thiazide-induced potassium depletion, which causes intracellular acidosis and can lead to hypokalemia and hypocitraturia (Moe, 2006). Potassium citrate is effective in the treatment of patients who have calcium stones and normal urinary calcium. By providing an alkali load, potassium citrate increases urinary pH and citrate, therefore mediating the inhibitory effects of macromolecular modulators of calcium oxalate crystallization. The major drawback for a more widespread use of alkali citrate preparations is the relatively low tolerability of available alkali citrate preparations. Adverse effects that reduce treatment compliance have been noted mainly in the gastrointestinal tract and include eructation, bloating, and diarrhoea (Mattle and Hess, 2005). In conclusion, none of the listed treatment modalities are without any side effects. Thus, the focus should be on the development of novel strategies for the prevention and treatment of kidney stone disease. Herbal medicines could close the gap in this regard.

2.2 Section B: Ethnobotany and its relevance in Primary Health Care and Drug Development

In the section, a brief review regarding the role of ethnobotany in the context of bioprospecting of indigenous medicinal plants was attempted. The term ethnobotany was coined by John W. Harsberger in 1896 (Davis, 1995) and was considered as the art of collection of useful plants by a group of people and the
description of the uses of plants. Over the last century, ethnobotany has evolved into a scientific discipline that focuses on the people-plant relationship in a multidisciplinary manner, incorporating not only collection and documentation of indigenous uses but also ecology, economy, pharmacology, public health, and other disciplines (Gomez-Beloz, 2002). At present, ethnobotany has become more and more valuable in the development of health care and conservation programs in different parts of the world (Balick, 1996). In India, studies on ethnobotany were initiated by Janaki Ammal as an official programme in the Economic Botany Section of Botanical Survey of India (Howrah) in 1954. From 1960, Jain started intensive field studies among tribal areas of central India (Jain 1963 a, b, c, d; 1964a). An All India Coordinated Research Project on Ethnobiology (AICRPE) came into operation from 1982 at NBRI, Lucknow, and four centers [Shillong, Howrah, Coimbatore and Port Blair] of Botanical Survey of India.

Ethnomedicine is a branch of ethnobotany which refers to the study of traditional medical practice which is concerned with the cultural interpretation of health, diseases and illness and also addresses the healthcare seeking process and healing practices often transmitted orally from generation to generation (Krippner, 2003). WHO experts have defined traditional medicine as “The sum total of all the knowledge whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance and relying exclusively on practical experience and observation handed down from generation to generation whether verbally or in writing” (1978). Ethnobotanical studies that explore and help to preserve knowledge are therefore urgently needed before traditional folklores are lost forever (Chaudhary, 1998).

The plant-based traditional medical systems keep on providing the primary health care to more than three-quarters of the world population. They depend on medicinal plants because of their effectiveness, cultural preferences and lack of modern healthcare alternatives (Caniago and Siebert, 1998). It is estimated that less than 10% of the word’s genetic resources have been studied seriously as sources of medicine. Until now, from this small fraction, humanity has achieved enormous benefits. In the developed countries 25% of the medical drugs are based on plants
and their derivatives (Principe, 2005). Today a significant number of drugs are developed from plants which are active against a number of diseases. The bulk of these involve the isolation of the active ingredient (chemical compound) found in a particular medicinal plant and its subsequent modification (Fabricant and Farnsworth, 2001). According to data released by the World Health Organization (WHO, 2003), ethno medicine has maintained its popularity in all regions of the developing world and its use is rapidly expanding in the industrialized, for example, in China traditional herbal preparation account for 30–50% of the total medicinal consumption. In San Francisco, London and South Africa, 70% of people living with HIV/AIDS use traditional medicine. Today the annual global market for herbal medicine stands at over US $60 billion (WHO, 2003). In addition, of the top 150 proprietary drugs used in the United States of America (USA), 57% contain at least one major active compound currently or once derived from plants (Grifo, 1997). International trade on medicinal plants is, therefore, increasing rapidly mainly as a result of intensified adoption of crude extracts for self-medication by the general public in the developed countries.

In India, the use of plants for medicinal treatment dates back to 5000 years. It was officially recognized that 2500 plant species have medicinal value while over 6000 plants are estimated to be explored in traditional, folk and herbal medicine (Huxley, 1984). In Ayurveda, for example, *Crataeva nurvala* is highly acclaimed for its use in the management of urinary tract disorders especially kidney stones. Texts date back from the 8th century BC record its application in urological diseases, with stronger emphasis on its use for kidney stones were recorded around 1100 AD (Deshpande et al., 1982). Its ancient status as the main Ayurvedic herb in urinary disorders is now supported by pharmacological and clinical researches. With the realization that indigenous medicines should form an indispensable part of health care, several organizations like the CSIR, ICMR, ICAR, many Universities and National Laboratories have been working vigorously to evaluate clinically the usefulness of local medicinal plants (Satyavati, 1982; Chopra et al., 1969; Satyavati et al., 1976).
2.2.1 Traditionally used Antiurolithiatic Medicinal Plants

An important prerequisite for the proper utilization of raw materials of the country is the survey of its natural resources and the preparation of an inventory. It is necessary that we should have full knowledge regarding the occurrence, frequency, distribution and phenology of various plants for their proper utilization. The present part of review of literature was attempted to point out the works so far done to identify traditionally used antiurolithiatic medicinal plants:

Cheryl (2006), made an investigation on Ethnomedicines used in Trinidad and Tobago, Canada, for urinary problems and diabetes mellitus based on ethnobotanical interviews conducted from 1996–2000. He reported the usage of the following fourteen plants for kidney and other urinary problems viz., *Kalanchoe pinnata*, *Mimosa pudica*, *Chamaesyce hirta*, *Flemingia strobilifera*, *Peperomia rotundifolia*, *Petiveria alliacea*, *Nopalea cochenillifera*, *Apium graveolens*, *Cynodon dactylon*, *Zea mays*, *Theobroma cacao*, *Lepianthes peltata*, *Eleusine indica*, *Gomphrena globosa*, *Pityrogramma calomelanos* and *Vetiveria zizanioides*. From Haramosh and Bugrote valleys in Gilgit of the Northern Areas of Pakistan, Khan and Khatoon (2007), had reported the use of *Juniperus communis* L. berries against kidney stone.

Several reports about the ethnobotanical use of medicinal plants for the treatment of urolithiasis are available from different states of India. According to the folk-medicine system of Kumaun Himalaya of Uttarakhand, India, *Bergenia ligulata* (Wall.) is useful in dissolving kidney and bladder stones (Gangwar et al., 2010). Prachi et al. (2009), had documented Medicinal plants of Muzaffarnagar district of Uttar predesh used in treatment of urinary tract and kidney stone. The information on medicinal uses is based on the exhaustive interviews with local healers and herbalists, practicing traditional system of medicine. The study revealed the use of *Abutilon indicum*, *Aerva lanata*, *Boerhaavia diffusa*, *Bryophyllum pinnatum*, *Crataeva nurvala*, *Cynodon dactylon*, *Daucus carota*, *Equisetum debile*, *Gomphrena celosioides*, *Musa balbisiana*, *Ricinus communis*, *Solanum surattense*, *Trianthema portulacastrum*, *Tribulus terrestris* and *Zea mays* as anti-urolithiatic agents in local remedies. Among this *Equisetum debile* and
**Gomphrena celosioides** are most effective and commonly used in treatment of urinary tract and kidney stones.

From Rajasthan two reports are available. Pareek and Trivedi (2011), had done a floristic survey of ethnomedicinal plants occurring in the tribal areas of Kaladera region in Rajasthan and reported the use of *Boerhaavia diffusa* leaves and *Commiphora wightii* gum resin for the treatment of gout and other forms of arthritis. Choudhary *et al.* (2008), had reviewed useful ethnobotanical information about the uses of plants by the tribals of Rajasthan as food, fodder, medicine, timber, fire-wood, tannin, dye, oil, fibre, alcohol, gum and resin. According to this study, *Ceropegia bulbosa* was effective for the treatment of urinary stones and other associated inflammation in urinary tract among the tribals of the study area.

From Manipur, extensive studies on traditional knowledge about kidney stones treatments are available. Mohd and Kumar (2011), reported traditional knowledge about the kidney stones treatment by Muslim Maiba (Herbalists) of Manipur, India. They had reported the use of *Emblica officinalis*, *Myriogyne minuta*, *Enhya fluctuans*, *Fragaria nilgerensis*, *Celtis timorensis*, *Cinnamomum tamala*, *Mentha arvensis*, *Bambusa nutans*, *Eupatorium spp.*, *Averrhoa carambola*, *Centella ssp.*, *Allium odoroum*, *Tamarindus indica*, *Oxalis corniculata*, and *Hedychium aurantiacum* for the treatment of kidney stone. Lokendrajit *et al.* (2011), conducted a study among different ethnic communities of Manipur and reported large number medicinal plants for the treatment of urolithiasis. The plants listed in the study are: *Centella asiatica*, *Cyperus rotundus*, *Desmodium microphyllum*, *Fragaria indica*, *Helianthus annuus*, *Polygonatum multiflorum*, *Rhus succedanea*, *Bonnaya reptans*, *Rubus niveus*, *Solanum nigrum*, *Stephanie hernandifolia*, *Tagetes erecta*, *Xanthium strumarium*, *Actinodaphne angustifolia*, *Cissus javana*, *Cordia grandis*, *Cuminum cyminum*, *Homonoia riparia*, *Pratia nummularia*, *Wedelia chinensis*, *Cissus adnata*, *Aeschynomene indica*, *Ananas comosus*, *Cinnamomum glaucescens*, *Averrhoa carambola*, *Berberis aristata*, *Celosia argentea*, *Celtis australis*, *Hibiscus sabdariffa*, *Allium odorum*, *Citrus latipes*, *Abrus precatorius*, *Duchesnea indica*, *Emblica officinalis*, *Hedychium coronarium*, *Mallotus philippensis*, *Myriogyne minuta*, *Plantago major*, *Potentilla*
anserina, Thunbergia alata, Abutilon indicum, Bauhinia acuminata, Blechnum orientale, Cinnamomum bejolghota, Costus speciosus, Crataeva nurvala, Crinum asiaticum, Enhydra fluctuans, Indigofera tinctoria, Ixora sub-sessilis, Mimosa pudica, Momordica dioica, Orthosiphon spiralis, Pavetta indica, Sesamum indicum, Sida acuta, Smilax ovalifolia, Benincasa hispida, Piper longum, Eupatorium birmanicum, Asparagus racemosus, Tamarindus indica and Oxalis corniculata.

Studies on some Ethnomedicinal plants from Talaja taluka of Bhavnagar district, Gujarat by Bhatt et al. (2000; 2002), had reported some plants (Corchorus depressus, Solanum surattense, crataeva nurvala, Pedalium murex, Ascarantha longifolia, Ocimum sanctum and Hackelochola granularis) which are useful for calculi cure.

Pandey et al. (2005), had made an investigation on medicinal plants in Satpura plateau of Madhya Pradesh and reported the use of Eupatorium spp., Hydrangea macrophylla and Boerhaavia diffusa for kidney stone treatment.

A few medicinal plants from different parts of Maharashtra were reported for the treatment of urolithiasis. Patil (2012), had reported the ethno medicinal uses of Celosia argentea and Ensete superbum for the treatment of kidney stones among the tribals of Satpura hill region in Nandurbar district of Maharashtra. In another study by Biradar and Ghorband (2010), reported the use of Enicostemma axillare and Tectona grandis fruits to treat kidney stone among the tribals of Nandad district of Maharashtra. Dnyaneshwar and Sharad (2012) had conducted ethnobotanical survey among herbal practitioners of Gond, Andh Kolam and Pradhan tribes of Kinwat forest range of Nand district of Maharashtra to collect information on the use of medicinal plants. They had reported the use of Diplocyclos palmatus, Tectona grandis and Tribulus terrestris for the treatment of kidney stone.

From the state of Tamil Nadu Nandagopalan et al. (2011), had made an attempt to identify medically important folklore plants frequently used by rural communities of sacred groves of Pudukkottai district. He had reported the use of Punica granatum, Musa paradisiaca and Solanum torvum for the elimination of
kidney stones. In another study, conducted among the ethnic groups of Villupuram district in the South Western Ghats of India, Sankaranarayanan et al. (2010), had reported the use of decoction of *Tribulus terrestris* fruit in combination with *Crataeva magna* stem bark internally in the case of urinary infection and kidney stone disorder. Jeeva et al. (2005), conducted a survey among the indigenous people of Kanyakumari district and reported that *Aerva lanata*, *Tribulus terrestris* and *Scoparia dulcis* as highly effective for the treatment of kidney stone. The use of *Scoparia dulcis* for the treatment of urinary calculi was also reported in another study (Jeeva and Femila, 2012) conducted among Nadars of Atoor village of Kanyakumari district, Tamilnadu, India.

From the state of Kerala, not much ethnobotanical studies been reported on antiurolithiatic medicinal plants. The use of *Arenga wightii* and *Emblica ribes* for treating urinary complaints among Kurichia tribe inhabiting Thirunelli forest of Wayanadu district was reported by Udayan et al. (2008). In another study, Rajith and Ramachandran (2010), reported the use of powdered seeds of *Ensete superbum* mixed with milk for urinary disorder and kidney stone among Kurichyas of Kannur district, Kerala. Augustine (2000) reported the uses of *Rotula aquatica*, *Scoparia dulcis* and *Ensete superbum* for the treatment of urinary disorders among the Malampandaram tribes of Kerala. The vast Ayurvedic literature claims a number of plants such as *Rotula aquatica* Lour., *Aerva lanata* (L.) Juss. ex Schult., *Boerhaavia diffusa* L., *Hygrophila schulli* (Buch.- Ham.) M. R. & S. M., *Tribulus terrestris* L., *Crataeva magna* (Lour.) DC., *Cocos nucifera* L., *Elettaria cardamomum* (L.) Maton, *Musa x paradisiaca* L. *Solanum torvum* Sw., *Cinnamomum verum* Presl, Prir., *Moringa pterygosperma* Gaertn., *Plectranthus amboinicus* (Lour.) Spreng., *Biophytum reinwardtii* (Zucc.) Klotzsch., *Ananas comosus* (L.) Merr., *Clitoria ternatea* L., *Indigofera tinctoria* L., *Ocimum tenuiflorum* L., and *Oldenlandia herbacea* (L.) Roxb. to be useful for the treatment of lithiasis (Kritikar and Basu, 1918; Warrier et al., 1996; Manilal, 2003). However, not much detailed studies have so far been done on these medicinal plants to elucidate their antiurolithiatic property. The works so far done
to assess the antiurolithiatic property is presented in the following session under appropriate heads.

### 2.2.2 Antiurolithiatic studies using medicinal plants

*In vitro* and *in vivo* studies are routinely employed for screening medicinal plants with respect to their antiurolithiatic property in inhibiting/assessing the nucleation, aggregation and growth inhibition of urinary stone constituents. The chemical analysis of kidney stones shows that most of the urinary stones are chiefly consists of calcium oxalate and calcium phosphate. Hence, most of the studies to assay or screen the antiurolithiatic property of medicinal plants were done by initial screening of the crude drugs by standardized *in vitro* gel method of crystallization (Henisch *et al.*, 1965; 1970; 1998) or by *in vitro* method of Boumann and Wacker (1980) or by *in vivo* rat model experimentations. The effect of *Crataeva nurvala* bark decoction on calcium oxalate urolithiasis induced by 3% glycolic acid has been studied in rats. The decoction showed significant activity in preventing the deposition of calcium and oxalate in the kidney by inhibiting the activity of the liver enzyme glycolic acid oxidase. Treatment with *Crataeva nurvala* bark decoction was reported to lower the levels of intestinal NaZ, KZ-ATPases (Varalakshmi *et al.*, 1990). Experimental studies carried out on *Crataeva nurvala, Tribulus terrestris* and *Dolichos biflorus* revealed the effectiveness of these plants in preventing the deposition of stone material on glass beads in the urinary bladder of rats (Pramod *et al.*, 1981). When all the three plants motioned in the study, were shown to dissolve phosphate type of calculi in an *in vitro* model, oxalate, uric acid and cystine stones were not dissolved by *Crataeva nurvala* and *Dolichos biflorus* extracts. However, uric acid and cystine stones were dissolved to some extent by *Tribulus terrestris*. Clinical studies carried out on *Crataeva nurvala* showed that it changes the urinary chemistry of patients thereby reducing the lithogenic potential (Deshpande *et al.*, 1982).

Results of the study conducted by Sailaja *et al.* (2011), suggest that *Tridax procumbens* L. can be an alternative or an adjunctive measure to other therapies to prevent CaOx stone formation. The protective effects of the plant against CaOx stone formation and hyperoxaluria induced oxidative stress may be attributed to its
saponin and flavonoid principles. This *in vitro* study revealed that the extract of *Tridax procumbens* as a good source of antioxidants.

Swathi *et al.* (2008), conducted *in vivo* antilithiatic studies of aqueous extract of *Tephrosia purpurea* on the excretion and deposition of various calculi forming constituents like calcium, oxalate, magnesium and phosphate in urine and kidney. The aqueous extract of *Tephrosia purpurea* was found to be effective in reducing the formation as well as dissolution of existing calcium oxalate and magnesium ammonium phosphate stones.

Chauhan *et al.* (2009), had conducted *in vitro* study by using single diffusion gel growth technique and reported the inhibition of struvite crystals in the presence of herbal extract of *Boerhaavia diffusa*.

The antilithiatic property of *Moringa oleifera* was reported by a number of researchers. Karadi *et al.* (2006) and Sachan (2012), had evaluated the effectiveness of alcoholic extract of *Moringa oleifera* on calcium oxalate urolithiasis *in vivo* in male Wistar albino rat model experiments. The increased deposition of stone forming constituents in the kidneys of calculogenic rats were significantly lowered by curative and preventive treatment using aqueous and alcoholic extracts. The results indicate that the root-wood of *Moringa oleifera* is endowed with antiurolithiatic activity. In another study, Jameel *et al.* (2010), had reported the antiurolithiatic activity of bark of *Moringa oleifera*. Aqueous extract of the bark of *Moringa oleifera* was administered orally and evaluated for its antiurolithiatic potential in albino rats of Wistar strains. The stones were produced in this study by zinc disc foreign body insertion in the bladder supplemented with 1% ethylene glycol in drinking water. The reduction in the weight of the stones was used as a criterion for assessing the preventive or curative antiurolithiatic effect of the bark of this plant. Two doses of extract for prophylactic and curative groups were used. In both groups the oral administration of the extract of bark of *Moringa oleifera* has resulted in significant reduction in the weight of bladder stones compared to the control group.

The antiurolithiatic activity of hydroethanolic extract of *Lawsonia inermis* leaves against calcium oxalate-type stones under *in vivo* conditions was reported by
Kore et al. (2011). *In vivo* studies on the effect of *Ammi visnaga* seeds on kidney stones revealed that its antilithiatic effect is mainly because of highly potent diuretic activity and amelioration of uraemia and hyperbilirubinemia (Khan et al., 2001).

The inhibitory effect of *Phyllanthus niruri* was studied on crystal growth, in a rat model of urolithiasis by introduction of calcium oxalate seed in the bladder of rats. The effect may be due to higher Levels of glycosoamino glycans incorporated into calculi (Freitas et al., 2002). *In vitro* studies in which calcium oxalate precipitation was induced by the addition of 0.1 M sodium oxalate to unfiltered urine samples from Wistar rats and normal humans in absence and presence of *P. niruri* extract may interfere with early stages of stone formation (Barros et al., 2003). In an earlier study, Campos and Schor (1999) investigated the *in vitro* effect of *Phyllanthus niruri* on a model of CaOx crystal endocytosis by Madin-Darby canine kidney cells. The extract exhibited a potent and effective nonconcentration-dependent inhibitory effect on the CaOx crystal internalization.

Fourteen patients with renal calculi and 16 patients with ureteric calculi have been treated with the herbomineral combination containing *Bergenia ligulata* and *Tribulus terrestris*. About 28.57% of patients with renal calculi and 75% patients with ureteric calculi passed their calculi completely and in other patients there was a marked or partial expulsion of calculi along with changes in shapes and sizes of calculi (Sannidi et al., 1997). In another study, the seeds of *Dolichos biflorus* and rhizomes of *Bergenia ligulata* were tested for their *in vitro* antilithiatic and anticalcification activity by the homogenous precipitation method. The extracts were compared with an aqueous extract of cystone (a marketed preparation) for their activities. Also a combination of the extracts of the two plants was tested. Extracts of *Dolichos biflorus* showed activity almost equivalent to cystone while *Bergenia ligulata* showed less activity and the combination was not as active as the individual extracts (Farooq, 2004). The effect of herbal extracts of *Tribulus terrestris* and *Bergenia ligulata* on growth inhibition of calcium oxalate monohydrate crystals was studied by Joshi et al., (2005) under *in vitro* conditions. The results of this study revealed that both plants are effective in reducing the growth and aggregation of calcium oxalate monohydrate crystals.
The effect of ingestion of 3 and 10 g of tamarind pulp (*Tamarindus indicus*) was studied in normal subjects and in stone formers. Tamarind intake at the dose of 10 g showed significant beneficial effect in inhibiting spontaneous crystallization in both normal subjects and in stone formers (Rathore *et al*., 1993).

*Costus spiralis* is extensively used in Brazilian folk medicine for expelling urinary stones. Aqueous extract of *C. spiralis* when used at a dose of 0.25 and 0.5g/kg/day for 4 weeks had significantly reduced the growth of calcium oxalate calculi in the urinary bladder of rats (Viel *et al*., 1994).

Antiurolithiatic activity of two compounds viz., 7-hydroxy 2L, 4’, 5L-trimethoxyis of L avone and 7-hydroxy-4L-methoxy isoflavone isolated from the heart wood of *Eysenhardtia polystachya* was studied in rats by observing calculus formation experimentally induced by zinc discs. A significant decrease in urinary stone size was observed in animals treated with these compounds (Perez *et al*., 2000).

The aqueous extract of *Melia azedarach* was studied against ethylene glycol induced nephrolithiasis in male albino wistar rats. The aqueous extract of *M. azedarach* reduced urinary calcium, oxalate, phosphate and elevated urinary magnesium levels and urine volume (Garimella *et al*., 2001).

The aqueous extract of the bark of *Raphanus sativus* was tested for its antiurolithiatic and diuretic activity. The urolithiasis was experimentally induced by implantation of zinc disc in the urinary bladder of rats. Significant decrease in the weight of stones was observed after treatment in animals that received aqueous extract in comparison with control groups. This extract showed an increase in the 24 h urine volume as compared to the control (Vargas *et al*., 1999). In another study (Selvam *et al*., 2001), revealed the antilithiatic activity of *Raphanus sativus* on implants of calcium oxalate crystals or zinc discs in the urinary bladder of rats.

*Aerva lanata* was subjected to in vivo urolithiatic rat model studies by Soundararajan *et al*. (2006) and Mohamed *et al*., (2009). Administration of *Aerva lanata* aqueous suspension (2g/kg body weight) to CaOx urolithic rats had reduced the oxalate synthesizing enzymes and thereby formation of CaOx stones in vivo.
According to them, increased intake of extract of this plant would be helpful in urinary stone prophylaxis.

A few studies on *in vitro* and *in vivo* antiurolithiatic property of *Asparagus racemosus* was conducted by Shashi et al. (2009) and Kumar et al. (2009). The ethanolic extract, significantly reduced the elevated level of calculogenic ions and elevated the concentration magnesium, one of the inhibitors of urinary crystallization. Reduction in the calcium (Titrimetric analysis) and phosphate (Colorimetric analysis) precipitation was also reported.

The antiurolithiatic efficacy of *Plectranthus amboinicus* with special reference to calcium oxalate stones was subjected to detailed *in vivo* experimentation by Jose and Janardhanan (2005). Atmani et al. (2003), reported that an extract from the herb *Herniaria hirsuta* L., a plant that traditionally used in Morocco for the treatment of lithiasis, promoted the nucleation of calcium oxalate crystals, increasing their number but decreasing their size. In a follow-up study the authors could demonstrate that *H. hirsuta* could block crystal binding to cultured renal cells (Atmani et al., 2004).

Mohamed et al. (2009), studied the inhibition of mineralization of urinary stone forming minerals by medicinal plants such as *Achyranthes aspera*, *Passiflora leschenaultia*, *Solena amplexicaulis*, *Scoparia dulcis* and *Aerva lanata*. As revealed in this study, increased intake of fruit juice and seed extract of these plants would be helpful in urinary stone prophylaxis.

Fan et al. (1999), studied the crystallization of calcium oxalate in undiluted urine of healthy males and reported that free citrate and a calcium citrate complex inhibit calcium oxalate crystallization. Inhibitory effect of lemon juice on *in vitro* crystallization of calcium oxalate was reported by Abdelkhalak et al. (2005). As revealed in the above study, the ingestion of the lemon juice seems to dissipate an effect of great quantity of citrates which in turn increases the excretion of oxalates. The studies conducted by Kulaksizoglu et al. (2008), further substantiate the effectiveness of lemon juice to inhibit the rate of CaOx crystal nucleation and aggregation.
The inhibitory effect of tamarind, tomatoes and tartaric acid on urinary stone crystallization was studied by earlier researchers (Singh et al., 1987; Thomas et al., 1988; Joseph et al., 2005 and Anasuya and Sasikala, 1990). These studies revealed complete disappearance of calcium oxalate crystal aggregates, fall in density in crystalluria, reduction in crystal size, decrease in excretion of oxalic acid, increase in excretion of phosphorus and increased inhibitory activity of urine towards crystal growth.

In a recent study, Yogender et al. (2009), reported the regulatory action of *Jasminum auriculatum* flowers on endogenous oxalate synthesis. He had studied the efficacy of the aqueous and alcohol extracts of *Jasminum auriculatum* flowers on calcium oxalate nephrolithiasis in male albino rats. As revealed in the study, aqueous and alcohol extract of *J. auriculatum* flowers significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. Increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by curative treatment using aqueous and alcohol extracts.

The effect of aqueous and butanol fractions of *Tamarindus indica* to inhibit initial mineral phase formation of calcium phosphate and growth of calcium oxalate monohydrate crystals was studied by Chaudhary et al. (2008).

The aqueous extract of the root-stalk extract of *Cynodon dactylon* was tested for its antiurolithiatic activity. The urolithiasis was experimentally induced by chronic administration of calculi producing diet for 42 days. Administration of aqueous extract significantly reduced the elevated urinary calcium, oxalate and other calculogenous stone formation in the kidney (Satish et al., 2009).

Ethanol extract of the roots of *Homonoia riparia* was tested for its antiurolithiatic activity against calcium oxalate and magnesium ammonium phosphate stones in male albino rats. Calcium oxalate stones were induced by feeding 3% glycolic acid along with normal feed and magnesium ammonium phosphate stones were induced by implantation of zinc discs in the urinary bladder. The ethanol extract of *Homonoia riparia* (2 g/kg/day, orally) was found to be effective in reducing deposition of calcium in the kidney of both prophylactic and
curative group animals. The extract was found to be effective in reducing the formation and also in dissolving the pre-formed magnesium ammonium phosphate type of stones (Prasad et al., 1997).

The efficacy of alcoholic extract of *Mimusops elengi* (Purnima et al., 2010) and *Pergularia daemia* (Vyas et al., 2011) on antiurolithiatic and diuretic activity was studied by earlier researchers. The reduction of stone-forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of the plant extracts. In a similar study conducted by Doddola et al. (2008), the leaf juice of *Sesbania grandiflora* showed significant antiurolithiatic activity against calcium oxalate-type stones and also exhibited antioxidant properties. In another study, the aqueous extract of *Pinus eldarica* fruit extract significantly inhibited the formation of calculi without diuretic activity (Hosseinzadeh et al., 2010).

In a recent investigation, Chauhan et al. (2011), studied the inhibitory effect of aqueous root extract of *Rotula aquatica* against struvite crystals by single diffusion gel growth technique. It was observed that the number, dimension, total mass, total volume, growth rate and depth of growth of struvite crystals decreased with increasing concentration of extract. The enhancement of dissolution and fragmentation of struvite crystals suggested the potential application of *Rotula aquatica* root extract for inhibiting struvite type urinary stones.

### 2.2.3 Antiurolithiatic Phytoconstituents

There are several phytochemicals which could be accountable for the antiurolithiatic effect. According to Arafat et al. (2008), flavonoids and triterpenes have a key role in preventing urolithiasis. It is also believed that saponins and tannins act as antiurolithiatic phytoconstituents (Doddola et al., 2008). Soundararajan et al. (2006), had reported the dissolution of calcium oxalate crystals due to effect of flavonoids (Kaempferol-3-rhamnoside and kaempferol-3-rhamnogalactoside), triterpenes (betulin) and tannins. It is also reported that saponin rich fractions of other plants like, *Herniaria hirsuta* act as a great inhibitor of calcium stone formation under *in vitro* and *in vivo model* studies (Fouada et al.,
Lupeol has been found to be efficient in reducing the risk of stone formation in animals by way of preventing crystal-induced tissue damage and dilution of urinary stone-forming constituents (Malini et al., 2000).

2.3 Section C: Urinary stone types and Crystallographic Techniques

A brief review of different urinary stone constituents and scientific advancements made in crystallographic techniques in recent years for analyzing these stone constituents are discussed below under appropriate headings.

2.3.1 Urinary stone types

Stones are most often classified into 5 common types based on their chemical components. Calcium-containing stones, especially calcium oxalate monohydrate (Whewellite), calcium oxalate dihydrate (Weddellite) and basic calcium phosphate (Apatite) are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10-15%, uric acid 3-10% and cystine 0.5-1%. The most common types are calcium oxalate and calcium phosphate or a combination of these two (Otnes, 1983; Dietrich, 1990; Williams and Wandzilak, 1989).

2.3.1.1 Calcium Hydrogen Phosphate Dihydrate (CHPD)

The biomineral Calcium Hydrogen Phosphate Dihydrate (CaHPO$_4 \cdot 2$H$_2$O) is a pliable material that grows and dissolves faster than most other calcium minerals, including other calcium phosphate phases, calcium carbonates and calcium oxalates. CHPD is a white, odorless, crystalline substance. Other common names for the substance are Di Calcium Phosphate Dihydrate (DCPD) or brushite. The last name honors the US mineralogist George Jarvis Brush (1831 – 1912), who first described the mineral in 1865. In nature, large deposits of CHPD/brushite can be found in guano-rich caves where it is formed by the interaction of guano with calcite and clay at a low pH. In humans, larger amounts of brushite can only be found in pathological calcifications like kidney stones, dental calculi, chondrocalcinosis or some carious lesions (Le Geros, 2001; Hesse and Heimbach, 1999; Le Geros, 1991; Brown et al., 1975).
CHPD crystallizes in a monoclinic lattice. The crystals are needlelike or prismatic to tabular and up to 2 cm long. They can also have a foliated appearance. CHPD is sometimes considered as least soluble calcium phosphate compound at pH-values < 4.2. However, based on thermodynamical data, Anhydrous Calcium Hydrogen Phosphate (DCPA) is even more stable (Pan and Darvell, 2009). The hardness of CHPD is 2.5; its density is around 2.328 g/cm$^3$.

Brushite has a plate-like morphology dominated by \{010\} faces (Legeros and Legeros, 1971). The structure within the \{010\} plane is composed of two corrugated rows of Ca$^{2+}$ and HPO$_4^{2-}$, that are offset in the <010> direction. Between these calcium and phosphate containing sheets, are layers of water molecules bound to the calcium ions above and below the \{010\} plane. The weaker bonding of the water molecules to one another creates a cleavage plane between the two water layers perpendicular to the \{010\} face. Within the crystal, each calcium ion is bonded to 8 oxygen atoms, six from neighboring phosphates and two from water molecules. Thus, at a step edge where oxygen atoms are not available from neighboring phosphates, it is likely that the calcium ion will complete its coordination by binding water or OH- groups from the solution. As a reminder that unfulfilled oxygen bonds exist on these edges, the step-edges are cut such that the CaO$_8$ coordination remains intact. Yet, it should be noted that the exact form of the hydrated step edge is unknown. As the crystal grows, the oxygen atoms from the solution will need to be removed (or rearranged) to accommodate the adsorbing HPO$_4^{2-}$ ion and thus dehydration is expected to be an important part of the activation barrier for growth and dissolution (Vandervoort and Hartman, 1991). But, because, two water molecules remain as part of the crystal structure, this effect may be expected to be smaller.

It is also interesting that the \{010\} faces are fully hydrated as part of the bulk crystal structure and thus the removal of tightly bound water at \{010\} surface is not a part of the activation barrier on this facet. In other words, the large surface area of this facet is due to low surface energy rather than the kinetic barriers associated with dehydration. And in fact, brushite has a relatively low interfacial energy of 4.5mJ/m$^2$ compared to other biominerals such as 8mJ/m$^2$ for apatite.
(Nancollas, 2006) or 13.1mJ/m² for COM (Wu and Nancollas, 1999). Single crystal XRD studies show that this water layer is crystalline, but not ice-like and does not impart order of water molecules into the solution as might be expected from ice (Arsic et al., 2004). The fully hydrated surface also suggests that proteins are less likely to bind strongly to these surfaces, which been observed experimentally (Hanein et al., 1993; Flade et al., 2001). This is likely to play a role in the resorption properties of brushite cements.

2.3.1.1 Growth behavior of Brushite without additives

The crystallization kinetics depend explicitly on the \( \text{HPO}_4^{2-}/\text{Ca}^{2+} \) ratio. Controlled set of experiments performed by Giocondi et al. (2009), at constant supersaturation, constant pH, constant ionic strength, and across two orders of magnitude in \( \text{HPO}_4^{2-}/\text{Ca}^{2+} \) ratio have shown that \( \text{HPO}_4^{2-} \) incorporation is rate-limiting and that the growth rate can be doubled in solutions with high \( \text{HPO}_4^{2-} \) to \( \text{Ca}^{2+} \) ratios.

2.3.1.2 Influence of additives on Brushite growth

There are several generic ways that adsorbents can affect growth. They can incorporate into the crystal, they can change kinetic coefficients, they can pin steps, and they can act as surfactants. Each of these alters the step kinetics in characteristic ways that allow the differing mechanisms to be distinguished (De Yoreo and Vekilov, 2003; Qiu and Orme, 2008).

2.3.1.2 Crystal growth by Gel Technique

A good number of crystals have been grown by different gel techniques. It was in 1896, the German chemist, Robert Edward Liesegang (1896; 1897; 1926), first observed the periodic precipitation phenomenon. He first observed it, when a few drops of silver nitrate solution were introduced in gelatine gel impregnated with potassium dichromate. Periodic precipitation of infinite rings was observed. The utilization of gel as a medium of crystal growth was put forward by Fisher and Simons (1926). However it did not evoke much interest of crystal growers and remained as unexploited. The gel growth technique became very popular due to the pioneering work of Henisch H.K. (1965; 1970), who gave an authentic and
narrative record of this method. Following him, a number of investigators have used this elegant and relatively easy method for growing perfect or defect less crystals. Nowadays this method has been employed to grow not only the inorganic crystals but also to grow biological crystals in vitro because of its similarity with biological environment (Liesegang, 1926).

The growth of Calcium Hydrogen Phosphate Dihydrate can be simulated in the laboratory by growing crystals in silica hydro gel medium. In the gel growth technique, the gel acts as a ‘three dimensional crucible’ which supports the crystals; at the same time yields to its growth without exerting major forces upon it. In the gel growth technique growth occurs due to reaction between two solutions in a gel medium or achieving super-saturation by diffusion in gel medium. Slow and controlled diffusion of reactants in gels can mimic the condition in body. Biocrystallization or bio-mineralization usually occurs in the slow and steady process in the soft tissues. Single diffusion gel growth technique provides the simplified in vitro model of the highly complex growth of urinary calculi in vivo. Growth of crystals with different morphologies is commonly found in bio-mineralization. The crystal growth by gel method provides simulation of synovial cartilage and other biological fluids (Natarajan et al., 1997). In the gel growth technique, by changing the growth conditions, crystals with different morphologies and sizes can be obtained.

2.3.1.2.1 Preparation of Hydrosilica Gel

The water glass (Sodium Meta Silicate) powder of AR grade is dissolved in de ionized water and by changing the hydrogen ion concentration (pH) of the solution; the desired gel can be prepared. The pH factor is an important parameter, which determines the rate of polymerisation and the speed of gel setting (Au-Pang, 1963). For maintaining the acidity or the hydrogen ion concentration, an acid in requisite concentration is added to the system. Acids commonly used to acidify the gel are nitric acid, hydrochloric acid, tartaric acid, acetic acid, oxalic acid, orthophosphoric acid and selenous acid. For growing the crystals of a good number of materials, the pH range 6 to 8 are found suitable; however the minute changes in the ambience affect the habit of the crystals. It is observed that the fresh gels
between a pH range of 6-8 are highly transparent in nature (Patel and Arora, 1976). After adjusting the pH of the mixture it is taken in the crystallization vessels for polymerization. Test tubes, U-tubes etc. are commonly used as crystallizers and are kept under controlled thermal condition for proper setting.

One of the most important factors affecting the hardness of the gel medium is the density of the sodium meta silicate solution. In almost all the cases, it is observed that the dense gels produce poor quality crystals. It is found that a minimum density is desired for getting good quality gels for growth purpose. It is observed that the range of densities in between 1.03 to 1.06 gm/cc yields better experimental results in many systems.

2.3.1.2.2 The mechanism of Gel Formation

The structure and properties of the hydro silica gel has been described in great detail (Patel and Arora, 1976). It is worth noting that the hydrosilica gel is the polymerized form of silicic acid. When Sodium Meta Silicate is dissolved in water, mono-silicic acid is produced due to the reaction. This is a reversal process and the by-product, which is the strong alkali NaOH remains in the solution. This is the reason for the alkaline habit of the solution. The mono-silicic acid liberates the hydroxyl ions and polymerizes. This process continues until the entire molecule becomes part of the three dimensional network. The oxygen silicon linkage is extremely strong and irreversible. The by-product resulting from the reaction is water and it accumulates on the top of the gel because it is lighter than the gel. This phenomenon is called syneresisi (Plank and Drake, 1947). The period of gellation is controlled by the pH value of the solution. Another important feature of gel is its abundance of pores. The matrices contain fine pores having different dimensions. The pore is usually of the order of a micrometer in size. The pores may behave as capillary for the transport of ions. X-ray studies of silica gel show that it has close resemblance with silica glass but with some inhomogeneities. The full structure and behaviour of the gel still remains to be unraveled.
2.3.1.2.3 The Structure and Properties of Gel

The gel is defined as a highly viscous two component semisolid system rich in liquid and having fine pores. These fine pores may allow the free passage of electrolytes and sustain nucleation. The gel medium works as a 'Smart' material i.e., sensitive to the minute changes in the environment. Gels are broadly divided into two: organic and inorganic. If water is used as the liquid it is called hydro gel. The various types of gels used in crystal growth experiments are hydrosilica gel (sodium meta silicate), agar-agar gel, carbohydrate polymer gelatin gel (resembling protein structure), clay gel, soap fluid, poly-acrylamide, hydroxide in water etc. The silica gel made out of sodium meta silicate (SMS) is often used because of its easy availability and better performance in growing many crystal compounds. In some particular cases organic gels are used and the selection of the gel depends entirely on the nature of the electrolytes involved (Blank et al., 1970).

2.3.1.2.4 Advantages of Gel Technique

For crystal growth, the gel technique holds the greatest guarantee due to several valuable characteristics of the technique which are as follows:

- The crystals can be observed practically in all stages of growth due to the action of gel as a transparent crucible.

- The gel medium prevents the convection currents and turbulence considerably and thus the crystals formed are defect free or perfect in nature.

- The gel medium remaining chemically inert and harmless, the gel framework acts like a three dimensional crucible in which the crystal nuclei are delicately held in the position of their formation and growth, thereby preventing damage due to the impact with either the bottom or the walls of the container.

- It forms three-dimensional structure by entrapping water. Thermodynamic considerations reveal that, as the growth is happening at ambient temperature, the grown crystals would have fewer defects and are nearly perfect in nature.
• The gel being soft and porous yields mechanical to the growing crystals. Since the gel reduces the speed of chemical reagents, crystals could be made to grow much larger sizes than, they were formed by a similar reaction in water or in molten stage by decomposition process (Patel & Arora, 1973).

• The gellation structure provides an ideal medium for the diffusion of reacting ions and can be used to keep the reacting ions separated until the desired reaction.

• Concentration of the reactants can be easily varied.

• The nuclei are distributed individually in the medium and thereby the effects of precipitate interaction are drastically diminished.

• The technique is highly economical when compared with other methods.

• The grown crystals can be harvested easily without damaging the crystal faces. It yields good quality crystals with less expensive equipment.

The technique is widely used by several investigators to grow crystals having a variety of properties. Even though the quality of the crystals grown in gel is good, the size is invariably small compared to other methods.

2.3.1.3 Characterization of gel grown crystals

2.3.1.3.1 X-ray Powder Diffraction (XRD)

A great deal of knowledge about the architecture of molecules is derived from studies on the diffraction of X-rays by crystals. This method was first used by Bragg in 1913. An X-ray incident upon a sample will either be transmitted, in this case it will continue along its original direction, or it will be scattered by the electrons of the atoms in the material. All the atoms in the path of the X-ray beam scatter X-ray. In general, the scattered waves destructively interfere with each other, with the exception of special orientations at which Bragg's law is satisfied. The phenomenon of diffraction occurs when penetrating radiation, such as X-rays, enters a crystalline substance and is scattered. The direction and intensity of the scattered (diffracted) beams depend on the orientation of the crystal lattice with
respect to the incident beam. Any face of a crystal lattice consists of parallel rows of atoms separated by a unique distance (d-spacing), which are capable of diffracting X-rays. In order for a beam to be 100% diffracted, the distance it travels between rows of atoms at the angle of incidence must be equal to an integral multiple of the wavelength of the incident beam.

An X-ray diffractometer (Rigaku, Japan) utilizes a powdered sample (Vijayan, 1988) a goniometer and a fixed-position detector to measure the diffraction pattern of unknowns. The powdered sample provides (theoretically) all possible orientations of the crystal lattice, the goniometer provides a variety of angles of incidence and the detector measures the intensity of the diffracted beam. The resulting analysis is described graphically as a set of peaks with percentage intensity on the Y axis and goniometer angle on the X axis. The exact angle and intensity of a set of peaks is unique to the crystal structure being examined. The X-ray diffraction method is most useful for qualitative, rather than quantitative analysis (although it can be used for both). The monochromator is used to ensure that a specific wavelength reaches the detector, eliminating fluorescent radiation. The resulting trace consists of a recording of Intensity Vs Counter angle (Ashour et al., 1994). The trace can then be used to identify the phases present in the sample. Diffracted data from many materials has been recorded in a computer searchable Powder Diffraction file (JCPDS file). Matching the observed data allows the phases in the sample to be identified.

2.3.1.3.2 Energy Dispersive X-ray (EDX)

It is a technique used for identifying the elemental composition of the specimen. The EDX analysis system works as an integrated feature of the scanning electron microscope (SEM), and cannot operate on its own without the latter (Schroder, 1998; Flewit and Wild, 2003). During EDX Analysis, the specimen is bombarded with an electron beam inside the scanning electron microscope. The bombarding electrons collide with the specimen atom's own electrons, knocking some of them off in the process. The EDX spectrum is a plot of intensity of X-rays against energy of the emitted X-rays. An EDX spectrum normally displays peaks corresponding to the energy levels for which most X-rays have been received.
Each of these peaks is unique to an atom, and therefore corresponds to a single element. The higher the intensity of peak in a spectrum, the more concentrated is the element in the specimen.

2.3.1.3.3 Thermal characterization- TGA/DTA analysis

Thermo gravimetric Analysis (TGA) is a type of thermoanalytical testing performed on materials to determine changes in weight in relation to changes in temperature. TGA is commonly employed in research and testing to determine characteristics of materials such as: degradation temperatures, absorbed moisture content of materials, the level of inorganic and organic components in materials, decomposition points of explosives and solvent residues. TGA relies on a high degree of accuracy in three measurements: weight, temperature, and temperature change. A simultaneous TGA-DTA measures both heat flow and weight changes in a material as a function of temperature or time in a controlled atmosphere. Simultaneous measurement of these two material properties not only improves productivity but also simplifies interpretation of the results. The complimentary information obtained allows differentiation between endothermic and exothermic events which have any associated weight loss (e.g., melting and crystallization) and those which involve a weight loss (e.g., degradation).

TGA analyser usually consists of a high-precision balance with a pan (generally platinum) loaded with the sample. The pan is placed in a small electrically heated oven with a thermocouple to accurately measure the temperature. The atmosphere may be purged with an inert gas to prevent oxidation or other undesired reactions. A computer is used to control the instrument. Analysis is carried out by raising the temperature gradually and plotting weight against temperature. The temperature in many testing methods routinely reaches 1000°C or greater, but the oven is so greatly insulated that an operator would not be aware of any change in temperature even if standing directly in front of the device. In Differential thermal analysis (DTA), the material under study and an inert reference are made to undergo identical thermal cycles, while recording any temperature difference between sample and reference (Joshi and Joshi, 2003). This differential temperature is then plotted against time, or against temperature (DTA
curve or thermogram). Changes in the sample, either exothermic or endothermic, can be detected relative to the inert reference. Thus, a DTA curve provides data on the transformations that have occurred, such as glass transitions, crystallization, melting and sublimation. The area under a DTA peak is the enthalpy change and is not affected by the heat capacity of the sample.

2.3.1.3.4 Scanning Electron Microscope (SEM)

The Scanning Electron Microscope (SEM) uses electrons rather than light to form an image (Schroder, 1998). Preparation of the samples is relatively easy since most SEMs the only requirement is that sample should be conductive. The SEM has a large depth of field, which allows a large amount of the sample to be in focus at one time. It also produces images of high resolution, which means that closely spaced features can be examined at a high magnification. The combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the most profoundly used instruments in current research and development. A loop of tungsten filament functions as the cathode and emits the electron beam. A voltage is applied to the loop, causing it to heat up. The anode, which is positive with respect to the filament, forms powerful attractive forces for electrons. This causes electrons to accelerate toward the anode. The anode is arranged, as an orifice through which electrons would pass down to the column where the sample is held. The streams of electrons that are attracted through the anode are made to pass through a condenser lens, and are focused to very fine point on the sample by the objective lens. The electron beam hits the sample, producing secondary electrons from the sample. These electrons are collected by a secondary detector or a backscatter detector, converted to a voltage, and amplified. The amplified voltage is then applied to the grid of the Cathode Ray Tube (CRT) that causes the intensity of the spot of light to change. The image consists of thousands of spots of varying intensity on the face of a CRT that corresponds to the topography of the sample.

2.3.1.3.5. Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy is a very useful technique for characterization of materials that gives information about the composition and the structure of
molecules. The advantages of infrared spectroscopy include wide applicability, non-destructiveness, measurement under ambient atmosphere and the capability of providing detailed structural information. FTIR has additional merits such as: higher sensitivity, higher precision, quickness of measurement and extensive data processing capability (Fately and Mc Devi, 1971; Halberstadt and Henish, 1968; Lippincott and Schroeder, 1955).

IR spectra originate in transitions between two vibrational levels of a molecule in the electronic ground state and are usually observed as absorption spectra in the infrared region. For a molecule to present infrared absorption bands the main requirement is that it has a permanent dipole moment. When a molecule with at least one permanent dipole vibrates, this permanent dipole also vibrates and can interact with the oscillating electric field of incident infrared radiation. In order for this normal mode of vibration of the molecule to be infrared active, i.e., to give rise to an observable infrared band, there must be a change in the dipole moment of the molecule during the course of vibration. If the vibrational frequency of the molecule, as determined by the force constant and reduced mass, equals the frequency of the electromagnetic radiation, then adsorption can take place. As the frequency of the electric field of the infrared radiation approaches the frequency of the oscillating bond dipole and the two oscillate at the same frequency and phase, the chemical bond can absorb the infrared photon and increase its vibrational quantum number by +1, or increases its vibrational state to a higher level. As the first approximation, larger the strength of the bond the higher the frequency of the fundamental vibration. In the same way, the higher the masses of the atoms attached to the bond the lower the wave number of the fundamental vibration. As a general guide, the greater the number of groups of a particular type and more polar the bond, the more intense is the band.

The infrared spectrum can be divided into two regions, one called the functional group region and the other the fingerprint region. The functional group region is generally considered to range from 4000 to 1500 cm\(^{-1}\) and all frequencies below 1500 cm\(^{-1}\) are considered characteristic of the fingerprint region. The fingerprint region involves molecular vibrations, usually bending motions that are
characteristic of the entire molecule or large fragments of the molecule (Srivastava et al., 1982; Stuart and Sutherland, 1956). Thus these are used for identification of the material. The functional group region tends to include motions, generally stretching vibrations, which are more localized and characteristic of the typical functional groups, found in organic molecules. While these bands are not very useful in confirming identity, they do provide some very useful information about the nature of the components that make up the molecule.

Basically an IR spectrometer is composed of the source, the monochromator and the receptor. An ideal IR source would be one that would give a continuous and high radiant energy output over the entire IR region (Sonin et al., 1968). The two sources in most common use are the Nernst Glower (heated up to 2200K) and the Globar (heated to about 1500K). In general, in all IR sources the radiant energy, which depends upon the temperature of the source, is low in the far infrared, and to obtain sufficient energy the slit width of the source has to be opened considerably with a corresponding decrease in resolution. Between the source and the detector there must be some kind of device to analyse the radiation so that intensity can be evaluated for each wavelength resolution element. There are two basic types, namely, monochromators, used in dispersive instruments, and interferometers used in Fourier transform instruments. In a monochromator, a prism or a diffraction grating is used, separating the components of polychromatic radiation. For spectroscopic work the prism must be transparent to the particular wavelength region of interest and the dispersion of the prism must be as large as possible. The final part of the spectrometer is the detector. The IR detector is a device that measures the IR energy of the source that has passed through the spectrometer. Their basic function is to change radiation energy into electrical energy, which can be generated to process a spectrum.

In the case of FTIR spectroscopy, the spectra are recorded in the time domain followed by computer transformation into the frequency domain, rather than directly in the frequency domain, as is done by dispersive IR spectrometers. To record in the time domain, interference has to be used to modulate the IR signal at a detectable frequency. This is done by means of the well known Michelson
interferometer, which is used to produce a new signal (interferogram) of a much lower frequency which contains the same information as the original IR signal. In the FTIR instrument, the sample is placed between the output of the interferometer and the detector. The sample absorbs radiation of particular wavelengths. Therefore, the interferogram contains the spectrum of the source minus the spectrum of the sample. An interferogram of a reference (sample cell and solvent) is needed to obtain the spectrum of the sample. After an interferogram has been collected, a computer performs a fast Fourier transform, which results in a frequency domain trace (i.e. intensity vs. wave number).

2.4 Section D: A Review of Medicinal Plants Selected for Antiurolithiatic Study

A brief review of the medicinal plants selected for in vitro growth dissolution studies was attempted with special reference to their traditional, phytochemical and pharmacological properties.

2.4.1 Achyranthes aspera L.

*Achyranthes aspera* L. (common name: Rough Chaff tree (English) belonging to the family Amaranthaceae) is widely distributed throughout India, Baluchistan, Ceylon, Tropical Asia, Africa, Australia and America (Anonymous, 2005; Gupta, 2010).

2.4.1.1 Traditional uses

Traditionally, the plant is used in asthma and cough. Crushed plant parts are boiled in water and are used in pneumonia. Infusion of the root acts as a mild astringent in bowel complaints. The flowering spikes/seed, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles and also for night blindness and skin diseases (Nadkarni, 2009). A fresh piece of root is used as tooth brush. Root Paste in water is used in ophthalmia and opacities of the cornea. Similarly, fresh leaves paste is used for allaying pain from bite of wasps (Gupta, 2010). Ash of the plant is applied externally for ulcers and warts. The crushed leaf rubbed on aching back is a remedy for strained back (Singh et al., 1996). For snake bites, the ground root is given with water until the patient vomits and regains consciousness. Inhaling the fume of *Achyranthes aspera*
mixed with *Smilax ovalifolia* roots is suggested to improve appetite and to cure various types of gastric disorders (Srivastav *et al*., 2011). The plant is useful in liver complaints, rheumatism, scabies and other skin diseases. It also possesses tranquillizing properties (Khare, 2007).

### 2.4.1.2 Phytochemical studies

Researchers like Hariharan and Rangaswami (1970) and Ali (1993), conducted chemical investigations on the seeds of *Achyranthes aspera* and identified Saponins A (D-Glucuronic Acid) and saponins B (β-D-galactopyranosyl ester of D-Glucuronic Acid). Along with these constituents they isolated certain other constituents’ viz., oleanolic acid, amino acids and hentriacontane. Besides, Ram *et al*., (2004) and Ali (1993), reported chemical constituents like 10-tricosanone, 10-octacosanone and 4-tritriacontanone from its seeds. Seshadri *et al*., (1981), isolated two constituents from the fruits and were identified as Saponins C and D. Kunert *et al*., (2000), isolated three bisdesmosidic saponins (I-III), 20-hydroxyecdysone, and quercetin-3-O-β-D-galactoside, from the methanol extract of the aerial parts of *Achyranthes aspera*. Their structures were established on the basis of NMR spectroscopic analysis; the complete H1 and C13 assignments of the compounds were achieved by means of 2D NMR studies. Michl *et al*., (2000), reported two new bisdesmosidic triterpenoid saponins besides the three known saponins from the methanolic extract of the aerial parts of *Achyranthes aspera*.

Studies conducted by Rameshwar & Akito (2007), revealed three oleonolic acid glycosides from the seeds of *Achyranthes aspera*. Chauhan *et al*., (2002), isolated a new cyclic chain aliphatic fatty acid from the seeds of this plant.

Khastgir *et al*., (1958), isolated sapogenin along with oleanolic acid from the seeds and roots of *Achyranthes aspera*. Ecdysterone was isolated from the methanolic extract of roots and other parts of *Achyranthes* by chromatographic studies in silica gel column (Banerji *et al*., 1970; Ikan *et al*., 1971). Sharma *et al*., (2009), isolated a new aliphatic acid, n-hexacos-14-enoic acid (first report from natural/synthetic source) from the ethanolic extracts of its roots. Dihydroxy
ketones such as 36, 37-dihydroxyhenpentacontan-4-one and Triaccontanol were isolated from the shoots of *Achyranthes aspera* (Batta and Rangaswami, 1973; Misra *et al*., 1991). Misra *et al*. (1993), reported certain long chain compounds like, 27-cyclohexylheptacosan-7-ol and 16-hydroxy-26-methylheptacosan-2-one from its aerial parts.

Neogi *et al*. (1970), reported Achyranthine, a water soluble alkaloid, possessing many pharmacological actions. Kapoor and Singh (1966), reported betaine (C$_{5}$H$_{11}$NO$_{2}$) from the whole plant which is also a water soluble alkaloid. Compounds like, Pentatriaontane, 6- pentatriacontanone, Hexatriaontane (Ali, 1993) and an aliphatic alcohol, Tritriacontane, 17-pentatriacontanol (Gariballa *et al*., 1983) were isolated from its shoots. Misra *et al*. (1996) isolated various compounds like tetracontanol-2 (C$_{40}$H$_{82}$O), 4-methoxyheptatriacont-1-en-10-ol (C$_{38}$H$_{76}$O) and $\beta$-sitosterol from *Achyranthes aspera*. Laddha *et al*. (2005), reported extraction, isolation and purification, characterization and quantification of 20-hydroxyecdysone from this plant. Rameshwar (2007), isolated chemical compounds of the volatile oil fraction from *Achyranthes aspera* leaves and were analyzed by GCMS. Seven compounds viz., pbenzoquinone, hydroquinone, spathulenol, nerol, $\alpha$-ionone, asarone and eugenol constituting 63.05% of the oil was identified. Among the volatile fraction, Hydroquinone (57.7%) was identified as the chief constituent.

### 2.4.1.3 Pharmacological actions

Anti-oxidant Activity of *Achyranthes aspera* was reported by a number of researchers (Tahiliani and Kar, 2000; Edwin *et al*., 2008; Gayathri *et al*., 2009; Malarvili and Gomathi, 2009). Analgesic and antipyretic activities of leaves, roots and seeds of *Achyranthes aspera* were reported by Sutar *et al*. (2008); Kumar *et al*. (2009) and Mehta *et al*. (2009). Studies conducted by Gokhale *et al*. (2002), Vetrichevan & Jegadeesan (2003) and Vijaya Kumar *et al*. (2009a) also confirmed the anti-inflammatory activity of *Achyranthes aspera*. In another study, Akhtar and Iqbal (1991) reported the hypoglycemic activity of this plant. According to Chakraborty *et al*. (2002), the methanolic extracts of leaves (alkaloid, nonalkaloid and saponin fractions) show cancer chemopreventive properties. In another study,
Bafna and Mishra (2004), studied the hepatoprotective activity against rifampicin induced hepatotoxicity in albino rats by using methanolic extract of the aerial parts of *Achyranthes aspera*. The nephroprotective activity of methanolic extract of the whole plant was also studied against lead acetate induced nephrotoxicity in male albino rats (Jayakumar *et al*., 2009). According to Barua *et al*. (2009), the methanolic leaf extract of *Achyranthes aspera* shows anti-depressant effects in forced swimming and tail suspension tests in mice and rats. Gupta *et al*. (1972) studied the effect of saponin from the seeds of *Achyranthes aspera* which showed significant diuretic effect in adult male albino rats. In another study, Goyal *et al*. (2007), reported the bronchoprotective effect of ethanolic extract of this plant in toluene diisocyanate (TDI) induced occupational asthma in Wistar rats.

According to Neogi *et al*. (1970), Achyranthine, a water-soluble alkaloid, isolated from *Achyranthes aspera*, has pharmacological properties to decrease blood pressure and heart rate, dilate blood vessels, and increase the rate and amplitude of respiration in dogs and frogs. Gupta *et al*. (1972a), had reported that, a mixture of saponins isolated from the seeds of *Achyranthes aspera* increased the force of contraction of the isolated heart of the frog, guinea pig and rabbit. The plant is also hypolipidemic (Khanna *et al*., 1992). Besides, the antimicrobial (Sharma *et al*., 2006; Khan *et al*., 2010; Prasad *et al*. 2009), antiparasitic (Bagavan *et al*., 2008; Zahir *et al*., 2009) spermicidal, anti-implantation and antifertility (Shibeshi *et al*., 2006; Paul *et al*., 2006, 2010; Vasudeva and Sharma, 2006) activities of this plant were also subjected to detailed evaluation.

### 2.4.2 *Aerva lanata* (L.) Juss. ex Schult.

*Aerva lanata* (L.) Juss. ex Schult. (Common name: Polpala; family: Amaranthaceae) is a prostrate to decumbent, sometimes erect herb, found throughout tropical India as a common weed in fields and wasteland (Krishnamurthi, 2003). It is widely distributed in Africa, Madagascar, Seychelles and other islands in the Indian Ocean and southern Asia from Arabia to India, Sri Lanka, Indo-China and Malaysia.
2.4.2.1 Traditional uses

*Aerva lanata* is useful in boils, cough and urinary calculi. In Ayurveda, *Aerva lanata* plant juice is recommended for all urinary diseases. Ayurveda recommends dried and powdered plant parts mixed with honey to suppress cough and sputum. This powdered drug processed with milk is recommended for heat/infected boils. Ayurveda recommends the drug, *Aerva lanata* with milk for spermatorrhoea, leucorrhoea and also to strengthen male and female reproductive systems. The plant is good for diabetes; anthelmintic, antilithiatic, demulcent, and also to cure cough, sore throat and wounds (Pullaiah, 2003). It is anti-inflammatory (Vertichelvan *et al*., 2000), diuretic (Udupihille and Jiffry, 1986) and nephroprotective (Shirwaikar *et al*., 2004). In folklore practice, hot water extract of *Aerva lanata* has been reported to be useful in diabetes mellitus (Vetrichelvan and Jegadeesan, 2002).

2.4.2.2 Phytochemical studies

Phytochemical analysis indicated that the leaf extract of *Aerva lanata* contains flavonoid glycosides, aervitrin, aervolane, aervoside, amyrin, betulin, campesterol, canthin-6-one, 10-hydroxy-canthin-6-one, carboline-1 propionic acid, chrysin, β-ecdysone, daucosterol, hentriacontane, narcissin, β-sitosterol, syringic acid, feruloyl tyramine and vanillic acid (Vetrichelvan and Jegadeesan, 2002).

Muthukumaran *et al*. (2011), conducted a preliminary phytochemical investigation of methanol and aqueous extracts of *Aerva lanata* aerial parts. Three principal bioactive compounds such as saponins, flavonoids and tannins were found in both methanol and aqueous extracts whereas; alkaloids were detected only in the methanol fraction. Zapesochnaya *et al*. (1992), had reported alkaloids viz., Canthin-6-one, β-carboline, aervine, methylaervine, aervoside and aervolanine from *Aerva lanata*.

Yamunadevi *et al*. (2012), studied the functional groups present in the crude powder of *Aerva lanata* stem, leaves, root and flower through FT-IR spectroscopy and confirmed the presence of amide, alcohols, phenols, alkanes, carboxylic acids, aldehydes, ketones, alkenes, primary amines, esters, ethers, alkyl
halides and aliphatic amines in flower extract. Compared to flower extract, in roots all the above functional groups except phenol and amides, in leaves all the above functional groups except ketones and primary amines and in stem all the above functional groups except alkenes and aldehydes were present.

2.4.2.3 Pharmacological actions


2.4.3 *Ensete superbum* (Roxb.) Cheesman.

*Ensete superbum* (family: Musaceae) is endemic to the Western Ghats, India, (Cheesman, 1968). The plant is known as ‘*kalluvazha*’ in Kerala, ‘*kalvazhai*’ in Tamil Nadu, ‘*kallubale*’ in Karnataka, ‘*rankeli*’ in Maharashtra and ‘*junglikela*’ in Gujarat.

This is a very hardy plant and prefers rocky barren areas. It is non-stoloneferous and does not produce suckers. Regeneration by seeds is the only natural means for propagation. *Ensete superbum* has enormous horticultural value in terms of being an ornamental and medicinal plant. It resembles a banana plant. The characteristic phyllotaxy on a large pseudostem gives *E. superbum*, a huge nested-fern like appearance. Its massive base and red flower head with broad leaves outlined in deep red and deep mid-rib are specific morphological features that add its appeal and aesthetic value.

A survey conducted by Saroj *et al.* (2010), in its natural habitat in Gujarat (Pavagadh, Dangs), Maharashtra (Veer, Raigad), Karnataka (Siddapur, Kargal) and Kerala (Ponmudi, Vithura and Kulathupuzha) revealed the threat to this medicinal plant due to excessive habitat degradation. Indiscriminate harvesting for commercial gains, grazing and destruction of immature fruits by elephants and monkeys have drastically reduced the population in the surveyed sites. This plant is

### 2.4.3.1 Traditional uses

Therapeutic potential of its seeds for various human ailments like diabetes (Satyavati, 1982), kidney stone (Yesodharan and Sujana, 2007), Leucorrhoea (Udayan et al., 2008), Measles (Patil and Bhaskar, 2006a), Stomachache and easy delivery (Jagtap, 2008) are widely reported. Anti fertility activity of *Ensete superbum* was reported by Fransworth (1975). According to Rajith and Ramachandran (2010) and Patil (2012) its powdered seeds mixed with milk is good for urinary disorders.

### 2.4.3.2 Phytochemical studies

Monica (2008), isolated a chroman derivate phytosterol - 4-hydroxy-3-methyl-hex-5-enyl from the seeds of *E. superbum*. It is used as a food additive to reduce cholesterol, and also as a cosmetic product. In another study, Kachroo and Agrawal (2010), proposed HPTLC method for the estimation of isolated chroman derivatives from the seeds. The detection and quantification were observed at wavelengths of 256 and 550 nm. HPTLC analysis indicated that the maximum amount of isolated derivative was present in ethanol fraction (1.83%) followed by ethyl acetate (1.74%) and methanol fraction (0.70%). The petroleum ether fraction of the extract was found to contain no traces of the isolated compound.

### 2.4.3.3 Pharmacological actions

According to Dutta *et al.* (1970), the fraction (VIDR-2GD) isolated from the seeds of Ensete *superbum* possess anti-implantation activity. According to Kachroo and Agrawal (2009), the anti-implantation and anti-estrogenic activity of Ensete *superbum* is attributed to its chroman derivative.
2.4.4 *Rotula aquatica* Lour.

*Rotula aquatica* Lour. (Family: Boraginaceae) is found scattered throughout India in the sandy and rocky beds of streams and rivers. The plant grows characteristically among rocks and trailing over gravel in stream beds. The plant is a small, much branched shrub, 60-180 cm in height with numerous short lateral arrested branchlets with rooting. Leaves are simple, nearly sessile, spathulate, rounded at the apex, more or less hairy, crowded on branches. Flowers are pink or reddish, shortly pedicellate, single or 2-3 together on short lateral branches, stamens exerted beyond the corolla tube. Fruits are subglobose, orange red drupes, tipped with the remains of the style (Warrier *et al*., 1996).

2.4.4.1 Traditional uses

Yesodharan and Sujana (2007), had reported the use of *Rotula aquatica* plant decoction for urinary complaints among the Malamalasar tribes of Kerala. Also, Udayan *et al*. (2007), reported its use among the Malapandaram tribes of Kerala for the treatment of body heating and prickles. The plant is also reported to be used for different ailments such as: urolithiasis (Diana *et al*., 2010) hyperglysimia, and for the treatment of tumour (Patil *et al*., 2003).

2.4.4.2 Phytochemical studies

The medicinal values of plant lie in their component phytochemicals such as alkaloids, flavonoids, phenolic compounds and other nutrients like amino acid, proteins, which produce a definite physiological action on the human body. The plant *Rotula aquatica* reported to contain baunerol, steroids, alkaloids and allantoin (Patil *et al*., 2003). A sterol named rhabdiol (C$_{35}$H$_{60}$O, m.p. 210º) has also been isolated from the roots (Pullaiah, 2006).

2.4.4.3 Pharmacological actions

Patil *et al*. (2004), had reported the antimitotic activity of root of *Rotula aquatica*. Anthelminthic potential (Singh *et al*., 2011, 2011a; Lakshmi *et al*., 2012) antioxident and antitumor properties of the plant was also reported (Patil *et al*., 2003). Pari *et al*. (2002), had reported the antidiabetic activity of the aqueous
extract of *Rotula aquatica* in alloxan induced diabetic rat and the antidiarrhoeal effect of alcoholic extract of *Rotula aquatica* was reported by Sunder *et al.* (2012).

Ashwini *et al.* (2008), screened anticrystal activity of *Rotula aquatica*, *Commiphora wightii* and *Boerhaavia diffusa* extracts against basic calcium phosphate, calcium pyrophosphate, and monosodium urate monohydrate. The effect of each plant was assayed on micro crystals in 24-well microplates *in vitro*. It was found that the aqueous extracts of all the three plants have growth inhibition activity, but only the aqueous extracts of *Rotula aquatica* and *Commiphora wightii* have the capacity to dissolve already formed crystals.

Mamta *et al.* (2010), experimentally evaluated the plant for antilithiatic activity in male albino rats by placing zinc discs of 4mm in the urinary bladder through an incision and sutured it using cat gut. The grouped animals were treated with petroleum ether, chloroform, ethyl acetate and aqueous extracts (1g/kg) for 4 weeks respectively and then, left untreated for 4 weeks. The 24 hr urine sample was collected and pH was determined one day prior to sacrificing. Zinc discs from individual animals were removed, washed, dried and weighed. The stones were analyzed qualitatively for calcium, oxalate, urate, cystine, magnesium, phosphate, ammonium and carbonate by standard analytical procedures. The results obtained showed the efficacy of aqueous extract in preventing stone formation and dissolving the preformed stones. Ethyl acetate extract showed moderate efficacy that may be attributed to diuretic action of allantoin. A significant decrease in magnesium and phosphate level indicated that, it was utilized for the stone formation. After treatment, the increased excretion of magnesium and phosphate was due to dissolution of formed stones by ethyl acetate and aqueous extracts.

Gilhotra *et al.* (2011), in their research article have mentioned that the alcoholic extract of the plant when administered orally for 28 days along with 1% ethylene-glycol (inducer of urolithiasis), increased urine volume, thereby reducing the tendency of crystallization. Histopathological studies on sections of kidney revealed that the microcrystal deposits of lithiasis were found in ethylene-glycol treated group and it was reduced after treatment with the extract.
2.4.5 Sphaeranthus indicus L.

The *Sphaeranthus indicus* of the family Asteraceae is much branched herb, strongly scented, annual, and erect with branched tap roots (Vikani *et al.*, 2008). Stems are cylindrical with toothed wings. Leaves are sessile, decurrent, 2–7 cm long, 1–1.5 cm wide, obovate-oblong, rounded or subacute, glandular-hairy, spinous-serrate or dentate, narrowed at the base and greenish-brown in color. Flowers are borne in terminal, solitary, globose, clusters of heads. Heads of flowers are purple, bracts are short slender and acuminate. In each head, the outer flowers are females, few or many, fertile, the central flowers bisexual, fertile or sterile, involucre narrow, bracts paleaceous, spatulate, acute, ciliate; receptacle small, naked. Corolla of female flowers are purple, slender, tubular, minutely two to three toothed; corolla of hermaphrodite flowers are purplish white, tubular or funnel-shaped, four to five toothed, anther-base sagittate, auricles acute or tailed, style-armed, filiform, sometimes connate. Fruits are oblong and have compressed achene in which pappus is absent.

2.4.5.1 Traditional uses

All the parts of the *S. indicus* have medicinal uses. In Ayurvedic system of medicine, the whole herb is used in insanity, tuberculous glands, indigestion, bronchitis, spleen diseases, elephantiasis, anaemia, pain in the uterus and vagina, piles, epileptic convulsions, asthma, leukoderma, dysentery, vomiting, urinary discharges, pain in the rectum, looseness of the breasts and hemicrania (Kirtikar and Basu, 1981). The whole herb is used in Ayurvedic preparations to treat epilepsy and mental disorders. Leaves dried in the shade and powdered are used in doses of 20 grains twice a day in chronic skin diseases as an antisyphilitic and a nervine tonic (Nadkarni, 2007; Prajapati *et al.*, 2003). Hot water extract of the herb is used as an anthelminitic, as a diuretic, as a fish poison and as an aphrodisiac (Paranjape, 2001; Chopra *et al.*, 1996).

Flowers are tonic, cooling and used in conjunctivitis (Chopra *et al.*, 1996) and give strength to weak eyes (Agarwal, 1997). The oil prepared using the plant root is reportedly useful in treating scrofula and as an aphrodisiac. The external application of the paste of this herb is beneficial in treating pruritus and edema,
arthritis, filariasis, gout and cervical adenopathy (Sahu, 1948). In unani, the herb is used as a tonic, laxative, emmenagogue, and also it increases the appetite, enriches the blood, lessens inflammation, cools the brain and gives luster to the eye, is good for sore eyes, jaundice, scalding of urine, gleet, biliousness, boils, scabies, ringworm in the waist, diseases of the chest. The plant is traditionally used for diarrhoea (Girach et al., 1994). Hot water extract of the entire plant is used for glandular swelling of the neck and for jaundice (Ikram, 1981). Ambavade et al. (2006), had reported the use of oil from the roots of S. indicus for the treatment of scrofula and as aphrodisiac. He also reported the use of herbal paste for the treatment of pruritis, oedema, arthritis, filariasis, gout, and adenopathy. Seeds and roots are stomachic and anthelmintic. Flower heads are used as blood purifier in skin diseases (Sadaf et al., 2006). He also reported the use of powdered leaves for the treatment of skin diseases, urethral discharges and jaundice. The leaves were also reported to have anxiolytic, macrofilaricidal, antimicrobial and insecticidal activities (Mishra et al., 2007; 2007a). According to Pande and Dubey (2007), S. indicus plant juice is effective for liver and gastric disorders.

2.4.5.2 Phytochemical studies

Sohoni et al. (1988), had isolated a sesquiterpene lactone, 7-hydroxyeudesm-4-en-6, 12-olide, and a sesquiterpene acid, 2-hydroxycostic acid, along with the known compounds, β-eudesmol and ilicic acid, from the acetone extract of S. indicus. Rojatkar and Nagasampagi (1992), had reported three 7-hydroxyeudesmanolides and two sesquiterpenoids, cryptomeridiol and 4-epicryptomeridiol from this plant. Pujar et al. (2000), had reported some Eudesmanoids such as 11α,13-dihydro-3α,7α-dihydroxy-4,5-epoxy-6β,7-eudesmanolide, 11α,13-dihydro-7α-acetoxy-3β-hydroxy-6β,7-eudesm-4-enolide and 3-keto-β-eudesmol have been isolated from S. indicus. Singh et al. (1988), had reported bicyclic sesquiterpene lactone from petroleum ether extract of aerial parts of S. indicus. Singh et al. (1989), had reported Isolation and characterization of sterol glycoside, the β-d-glucoside of (24S0)-24-ethylcholesta-4, 22-dien-3-β-ol.

Mishra et al. (2007), had isolated and reported a flavanoid C-glycoside, namely, 5-hydroxy-7-methoxy-6-C-glycosylflavone from the aerial part of S.
The plant is reported to contain deep cherry colored essential oil having methyl chavicol, d-cadinene, \( \alpha \)-ionone, p-methoxycinnamaldehyde, \( \alpha \)-terpinene, citral, geraniol, geranyl acetate, \( \beta \)-ionone, oscimene, eugenol, sphaeranthene, sphaeranthol, estragole, Indicusene (Baslas et al., 1959; Lodha et al., 2003).

Basu and Lamsal (1946), had reported an alkaloid sphaeranthenine from S. Indicus. Yadava and Kumar (1998), had reported Carbohydrates such as arabinose, galactose, glucose, fructose, lactose, maltose, raffinose and rhamnose from leaves of \( S. \) indicus. Yadava and Kumar (1999), had also reported a novel isoflavone glycoside, 5, 4'-dimethoxy-3'-prenylbiochanin 7-O-\( \beta \)-d-galactoside from leaves.

Shekhani et al. (1990, 1991), had isolated Eudesmenolide type of sesquiterpene glycoside, sphaeranthanolide, with immunostimulant potential from the flowers of \( S. \) indicus and also Eudesmenolides such as frullanolide, 11-alpha-13-dihydro: 3, alpha-7-alpha-dihydroxy: frullanolide, 11-alpha-13-dihydro from flowers. Gupta et al. (1967), had reported to contain stigmasterol and \( \beta \)-sitosterol in the alcoholic extract of powdered caputula. Yadav and Kumar (1998), had isolated a flavone glycoside, 7-hydroxy-3’, 4’, 6-tetramethoxy-flavone 7-O-\( \beta \)-d-(1-4)-diglucoside from the stem of \( S. \) indicus.

### 2.4.5.3 Pharmacological actions

The plant is analgesic and antipyretic (Nanda et al., 2009), antihyperglycemic (Dhar et al., 1968; Prabhu et al., 2008; Rajeev et al., 2010; Ramachandran et al., 2011), hepatoprotective (Tiwari and Khosa, 2009; Nayak et al., 2007), antioxidant (Shirwaikar et al., 2006; Tiwari and Khosa, 2009; Ramachandran et al., 2011), immunomodulatory (Bafna and Mishra, 2004a), anti-inflammatory (Basal and Jain, 2003; Nanda et al., 2010), antimicrobial (Shaikh et al., 1986; Dubey et al., 2000; Vijaya and Anathan, 1993; Naqvi et al., 1998; Garg and Kasera, 1982, 1983; Lalla et al., 2005; Dhar et al., 1968; Duraipandiyan et al., 2009; Vimalanathan et al., 2009), Skin disease (Sadaf et al., 2006), bronchodilatory (Sarpate et al., 2009), antihyperlipidemic (Pande and Dubey, 2009; Ramachandran et al., 2011), renoprotective (Srinivasaan et al., 2008), sedative (Galani and Patel, 2009), larvicidal (Hameed and Shah, 2003; Tiwari and Saxena, 2003; Nisha et al.,
2007), nematocidal (Sharma and Saxena, 1996; Ali et al., 1991), and vasodilatory (Srivastav et al., 1971).

2.5 Summary

The wide arrays of studies covered in this review clearly indicate the need for documentation of traditional knowledge on antiurolithiatic medicinal plants of Kerala. The review also highlights the importance of crystallographic assay of urinary stone constituents especially CHPD, based on gel method of crystallization and their characterization by FTIR, XRD, SEM/EDX and TGA/DTA. The review of literature helped the investigator not only obtaining a broad picture of the research area, but also to assimilate the basic essentials needed for the present study. The literature survey also helped the investigator to formulate basic hypotheses, design appropriate experimental strategies and to interpret the findings of the study under focus.