Urinary stone disease is a very serious disease affecting humans, since ancient times. It has been defined as the presence of one or more calculi in any location within the urinary tract (Hossein et al., 2010). It can cause serious medical consequences. The incidences of kidney stones have increased in western societies, this has been linked to lifestyle changes and economic development (Saisindhu et al., 2012).

The incidence of urinary tract stone disease is increasing. According to the National Health and Nutrition Examination Survey, as of 2012, 10.6% of men and 7.1% of women are affected by renal stone disease, compared to just 6.3% of men and 4.1% of women who were affected in 1994. One of the more striking new trends appears to be the increased incidence of stone formation in women. The increase in incidence of women affected by urinary tract stone disease has outpaced that of men. Although nephrolithiasis continues to be more common in men, the incidence rate ratio of men to women with urinary tract stones has narrowed from 3.4 to 1.3. One hypothesis for the disproportionate increase in stone disease in women is related to obesity. A significant positive correlation exists between obesity and nephrolithiasis for both genders; however, obese females are more likely to develop stones than obese males this may explain the decrease from 1994 to 2012 in the male-to-female ratio for stone formers (Raudakova and Monga, 2014).

Calcium stone formation is a complex process that involves numerous metabolic, anatomical and physio-pathological mechanisms. Occurrence of supersaturated states of crystal forming factors with the capacity to precipitate in urine are a key factor in calculus formation. The saturation state of a substance is expressed as the ratio between a given substance and its solubility variable (Worcester and Coe, 2010). The stone formation requires supersaturated urine. Super-saturation also depends on urinary pH, ionic strength, solute concentration and complexations (Pareta et al., 2011).
2.1. The Urinary System

2.1.1. Anatomy and physiology of kidneys

2.2. Kidney stone

2.2.1. Epidemiology

2.2.1.1. Intrinsic factors

2.2.1.2. Extrinsic factors

2.2.2. Signs and symptoms

2.3. Types of kidney stones

2.3.1. Calcium stones

2.3.2. Uric acid stones

2.3.3. Struvite stones

2.3.4. Cysteine stones

2.3.5. Xanthine stones

2.4. Inhibitors of stone formation

2.4.1. Inorganic inhibitors

2.4.1.1. Citrate

2.4.1.2. Magnesium

2.4.1.3. Pyrophosphates

2.4.2 Organic inhibitors

2.4.2.1. Osteopontin (Uropontin)

2.4.2.2. Urinary prothrombin factor I (UPTF I)

2.4.2.3. Tamm- horsfall Protein (THP)

2.4.2.4. Glucosaminoglycans (GAGs)

2.4.2.5. Renal lithostathine

2.4.3. High urine volume
2.5. Promoters of lithiasis

2.6. Mechanism of formation of renal stones
   2.6.1. Crystal nucleation
   2.6.2. Crystal growth
   2.6.3. Crystal aggregation
   2.6.4. Crystal retention

2.7. Diagnosis of kidney stones
   2.7.1. Laboratory tests
   2.7.2. X-Ray
   2.7.3. Ultrasound
   2.7.4. Computerized Tomography (CT Scan)
   2.7.5. Intravenous Pyelogram (IVP)
   2.7.6. CT urography

2.8. Management of stones
   2.8.1. Drug therapy
   2.8.2. Extracorporeal Shock Wave Lithotripsy (ESWL)
   2.8.3. Uteroscopy
   2.8.4. Percutaneous Nephrolithotomy (PNL)
   2.8.5. Open surgery

2.9. Medicinal plants
   2.9.1. Significance of weeds
   2.9.2. Phytochemical and antioxidants
2.10. Medicinal plants analysed in the study

2.10.1. Tribulus terrestris

2.10.2. Aerva lanata

2.10.3. Scoparia dulcis

2.10.4. Tridax procumbens

2.1. The Urinary system

The organs, tubes, muscles, and nerves that work together to create, store, and carry urine are the urinary system. The urinary system includes two kidneys, two ureters, the bladder, two sphincter muscles, and the urethra. The urinary system works with the lungs, skin, and intestines all of which also excrete wastes, to keep the chemicals and water in the body in a balanced state. (http://www.kidneyurology.org/Library/Urologic_Health.php/Urniary_system_and_ how_works.php).

Figure 1

Urinary system
2.1.1. Anatomy and physiology of kidneys

In humans, the kidneys are located in the abdominal cavity, more specifically in the para vertebral gutter and lie in a retroperitoneal position at a slightly oblique angle. Kidneys are positioned, one on each side of the spine. The asymmetry within the abdominal cavity caused by the liver typically results in the right kidney being slightly lower than the left, and left kidney being located slightly more medial than the right (Graff, 2002).

**Figure 2**

*Anatomy of kidney*

The kidney has a bean-shaped structure; approximately 11–14 cm in length, 6 cm wide and 4 cm thickness. Each kidney has a convex and concave surface. The concave surface, the renal hilum, is the point at which the renal artery enters the organ, and the renal vein and ureter leave. The kidney is surrounded by tough fibrous tissue, the renal capsule, which itself is surrounded by perinephric fat, renal fascia and paranephric fat. There are three major regions of the kidney, which includes renal cortex, renal medulla and renal pelvis. The outer granulated region is the renal cortex, consisting of about one million blood filtering nephrons, the filtration units of the kidney. It stretches down in between a radially striated inner layer, the renal medulla. This contains the pyramid shaped renal pyramids separated by renal columns. The ureters are continuous with the renal pelvis (Mader, 2004).
The main function of the urinary system is to maintain homeostasis through conserving water and electrolytes with a secondary function of excreting toxins. It is composed of kidneys, ureters, bladders and urethra. The kidneys are responsible for the primary filtering and rebalancing organs of the body. The ureters are conduits to the bladders from the kidneys. The bladder stores the urine until its disposal (https://mcb.berkeley.edu/courses/mcb135e/kidneys).

In addition, the urinary system plays a major role in maintaining blood volume, normal blood pressure, normal blood composition, maintaining the correct osmolality, ion concentration and acid-base status of the body. It also synthesizes and secretes calcitrol, the hormonally active form of vitamin D used by the body to regulate the blood levels of calcium and erythropoietin, a hormone that promotes the formation of red blood cells in the bone marrow. The function of kidney is to filter and regulate waste, solutes, toxins, proteins, amino acids, water, electrolytes and bicarbonates (Mckinley and O’ loughlin, 2011).

The kidneys absorb everything except the blood cells that pass through them and filter them out. This involves a two-step process. In step one, filtration of the plasma also known as ultrafiltration from the glomerular capillaries to the bowman’s capsules to form tubular fluid. The glomerular capillaries prevent the plasma proteins from passing through the nephrons. The filtration is non selective as all the components which the body needs to retain and excrete are filtered. In step two, based on the signals received from the brain and body, they reabsorb the necessary components in required amounts so as to achieve balance. The signals are received from receptors that monitor everything from dissolved bio-chemicals to blood pressure, from pulse to temperature (http://www.mf.uni-mb.si/mf/instituti/fizio/Seminars/seminar_17.pdf).

2.2 Kidney stone

Kidney stones are stone-like lumps that can develop in one or both of the kidneys. The medical terminology for stones in the kidneys is nephrolithiasis. The prevalence and incidence of nephrolithiasis is reported to be increasing across the world. Lifestyle changes dramatically influences stone formation (Romero et al., 2010).
2.2.1 Epidemiology

Humankind is known to be affected by stone diseases which was first reported in Egyptian mummies dated 4000 BC and reference being made in early Sanskrit documents in India between 3000-2000 BC (Prasad et al., 2007). The prevalence rate of the stone disease is 2-20% and recurrence rate of 50% (Butterweck and Khan, 2009). In the last few years, there has been a raise in the incidence of urinary stone disease with increase in the number of pediatric cases being reported (Johri et al., 2010). Researchers predict that due to global warming, by 2050 there will be a 30% growth in kidney stone cases (Fakheri and Goldfarb, 2011).

2.2.1.1 Intrinsic factors

Genetics

Genetic links to urolithiasis have been long established in certain heritable disorders, such as primary hyperoxaluria and the AGXT (Alanine-glyoxylate aminotransferase) gene; cystinuria and the SLC3A1 and SLC7A9 (Solute Carrier family) genes; and xanthinuria and the XDH (xanthine dehydrogenase) gene (Cellini et al., 2012; Eggermann et al., 2012). Having a family history of kidney stones doubles a person’s risk for the condition. Researches have been carried out to identify genetic markers and other factors that might predict the risk of kidney stones. These studies have implicated genes encoding the calcium-sensing receptor (CASR), osteopontin (OPN), vitamin D receptor (VDR), and the claudin family of genes (particularly CLDN14) in calcium urolithiasis (Vezzoli et al., 2010; Sayer, 2011). A family history of gout may also make a person prone to kidney stone (Ferrero et al., 2013).

Gender and age

Men are more prone to kidney stones than women with an estimated lifetime risk of renal stone of 10-20% in men and 3-5% in women because of the enhancing capacity of testosterone and inhibiting capacity of oestrogen. Another factor is because of larger muscle mass of men when compared to women.
Daily breakdown of tissue resulting in increased metabolic waste resulting in concentration of urine with calculogenic factors. The risk of kidney stones increases in men in their 40s and continues to rise till the age of 70 (Jyothi et al., 2012).

The risk of kidney stones in women peaks at the age of 50. In younger women, stones are more likely to develop during the late stages of pregnancy. It has become more common in children over the past few decades as a result of rapid variation in habits and increasing affluence (Raudakova and Monga, 2014).

**Ethnicity**

The risk of stone disease is significantly lower in Asians, African-Americans and Mexican when compared to Caucasians, due to difference in climatic conditions, diet, lifestyle. This might be due to the prevalence of higher rates of diabetes and high blood pressure (Akoudad and Kottgen, 2010).

**Body mass index/ weight**

The prevalence and incidence of calcium oxalate stone disease was directly associated with body mass index and weight gain. Obesity has been linked to hypertension, reduction of urinary pH and other metabolic changes like increased urinary oxalate excretion and increased uric acid production, which favor stone formation. A significant positive correlation was established between obesity and nephrolithiasis for both sexes, having a great impact on women than in men (Daudon et al., 2006).

**Medical conditions**

Medical conditions such as gout, high blood pressure, inflammatory bowel disease, urinary tract infection, hyperparathyroidism increases an individual’s risk of kidney stone disease (Blackwood et al., 2001).

2.2.1.2 Extrinsic factors

**Geography**

Geographical variation in diet, minerals in local water and rate of obesity plays a vital role in stone formation. The stone belts of the world are situated in
the countries of Middle East, North Africa, Mediterranean regions, North-western states of India and Southern states of USA. Socio-economic conditions and changes in dietary habits shows that the probability of stone formers varies in different parts of the world: 5-9% Europe, 12% Canada, 13-15% USA and 20% Saudi Arabia (Aggarwal et al., 2014).

Climate

High temperatures are correlated to stone formation. Increase in temperature leads to lower urine volume and supersaturation of urine with stone forming constituents, due to dehydration. One alternative reason may be the increased levels of vitamin D due to higher exposure to sunlight in warmer climate leading to concentration of urine with calcium (Fakheri and Goldfarb, 2009). Vitamin D is important in maintaining calcium homeostasis (Tang and Chonchol, 2013).

Occupation

Heat exposure and dehydration constitutes occupational risk factors for stone disease in manual workers. Heat exposure resulted in lower urine volume and pH and higher uric acid levels. People with sedentary occupation also posed increased risk of stone formation as limited activity can cause your bones to release more calcium (Sandhya et al., 2010).

Water

Increased fluid intake as a means of preventing recurrence of stone is well recognized today. In general, fluid intake of more than two liters per day is recommended for all patients, since this decreases saturation states by making urine more diluted and lowering the concentrations of crystallizable substances. Low-mineral water, especially with low sodium and calcium levels, is recommended for consumption (Bharathi and Amrithaveni, 2008).

Signs and symptoms of kidney stone disease

A kidney stone does not usually cause symptoms when it remains in the kidney. There, they can sometimes become infected leading to serious kidney infection called pyelonephritis. When the kidney stone passes from the urine
collecting system within the kidney into the ureter, it prevents easy flow of urine from
the kidney into the bladder, causing increase in pressure and swelling in the kidneys.
Pain from a kidney stone can be excruciating, particularly when the stone is passing
through the ureter. Kidney stone pain of this type is referred to as renal colic. The pain
often begins in the back or flank of the side of the low back. It may radiate to the front
of the abdomen and, in males, may cause testicular or scrotal pain. The pain can also
cause nausea, vomiting, and sweating (Kumar et al., 2012a).

The intense pain can be continuous or it can wax and wane as the stone
passes toward the bladder. Once the stone passes into the bladder, the
obstruction is relieved, urine can flow freely and the pain resolves. The dull flank
ache can remain for a few hours or days after the stone has passed. Since the
urethra is much wider than the ureter, passing the stone while urinating is usually
not an issue and most patients cannot tell when they have eliminated the stone
from their bladder. The quantity and severity of pain is not related to the size of
the stone but rather the amount of obstruction and kidney swelling present.
Sometimes, there can be blood visible in the urine as the kidney stone passes
and irritates the lining of the urinary tract. Most often, the urine is clear to the
naked eye and red blood cells are only visible in the urine when it is analyzed
under the microscope (Curhan et al., 2011).

2.3 Types of kidney stones
Calculi can be broadly classified into two main types namely

- Calcareous (calcium containing) – Calcium oxalate (80%) and calcium
  phosphate (5-10%).
- Non-calcareous stones- Uric acid (10%), Struvite (15-20%), cystine (1%),
  Xanthine stones.

Calcareous stones are usually visible on radiographic imaging whereas
non-calcareous stones are radiolucent and poorly visible on plain film
radiography (Peitrow and Karellas, 2006).
2.3.1 Calcium stones

More than 80% of all the kidney stones are composed of calcium usually combined with oxalate or oxalic acid also known as mulberry. A small percentage is made of calcium phosphate known as brushite. It is hard, single and has irregular shape or spikes. These spikes causes hematuria (blood in the urine) resulting in deposition of blood over stone giving it a dark appearance (Dawson and Tomson, 2012).

Crystal polymorphism is a well known phenomenon in nature. About 70% of kidney stones are composed of CaOx. A study of over 10,000 kidney stones from patients has shown that calcium oxalate monohydrate (COM) occurs about twice as frequently as calcium oxalate dihydrate (COD), although many stones contain both crystal forms. Asymptomatic crystals in urine are a common occurrence, even in many individuals who do not form stones, and these crystals are usually COD (Coe et al., 2005).
COD crystals are thermodynamically unstable and develop only under kinetically favorable conditions such as a high degree of supersaturation (high calcium concentration and/or hyperoxaluria), low concentrations of crystallization inhibitors (phytate, citrate), and urodynamically appropriate conditions (e.g., urinary stagnation). Due to thermodynamic instability, COD crystals slowly transform to stable COM crystals mainly in contact with urine. COM calculi, in which crystals are directly formed from urine, COM is known to have affinity for renal tubule cell surfaces (Grases et al., 2015).

Factors influencing calcium stone formation

- Hypercalciuria: Excessive calcium concentration in the urine due to high calcium absorption by the intestine (genetic factors), excessive chloride, renal calcium leak and excessive sodium in the urine (Ywen et al., 2010)
- Hyperoxaluria: Increased concentration of oxalate in the urine. It can be of either primary or secondary type. Primary oxaluria is an inherited disorder which leads to excess excretion of oxalate in the urine. Secondary oxaluria is caused by surplus dietary intake of oxalate or by problems in body’s breakdown of oxalate (Hoppe et al., 2009).
- Hypercalcemia: Excessive calcium in the bloodstream. It manifests due to the following reasons namely hyperparathyroidism, immobilization and renal tubular acidosis.
- Hyperuricosuria: High levels of uric acid in the urine causes calcium oxalate nephrolithiasis by promoting the formation of monosodium urate or uric acid crystals, which either act as seed crystals for calcium oxalate or adsorb normally occurring macromolecular inhibitors of calcium oxalate crystallization (Grover et al., 2003).
- Hypocitraturia: Reduced levels of citrate in the urine is responsible for removing excess calcium; Caused due to renal tubular acidosis, potassium and magnesium deficiency, urinary tract infection, kidney failure and chronic diarrhea.
- Low levels of kidney stone inhibitors (Sakhaee et al., 2011; Moe, 2011).
2.3.2 Uric acid stones

Uric acid stones are formed by the breakdown of purine, nitrogenous compounds found in the body and certain food products. It comprises of 10% of all kidney stones. The uric acid enters the blood stream and passes into the kidneys where they are expelled in the urine. Uric acid stones occur in acidic urine. These are multiple, small hexagonal, yellow to light brown colored. Uric acid stones are often found with calcium stones which make them opaque (Grases et al., 2006a; Tiwari et al., 2012).

Causes for uric acid stones are

- High uric acid in the urine for long period of time
- Volume of urine is Lesser than normal levels of urine produced
- Hyperuricosuria

2.3.3 Struvite stones

Struvite stones also known as phosphate stone amounts to 15% of the cases. It consists of triple phosphate of calcium, magnesium and ammonium. These are smooth and round in shape and are dirty white to yellow in colour. These stones are usually associated with urinary tract infection. The stones occur in the renal pelvis and grow in alkaline urine. Using the enzyme urease, these organisms metabolize urea into ammonia and carbon dioxide. This alkalinizes the urine, resulting in favourable conditions for stone formation. As it enlarges in the pelvis they slowly form the staghorn calculus. This stone is common in women and causes recurrent urinary tract infection, hematuria and damages the renal parenchyma (Vijaya et al., 2013).

2.3.4 Cystine stones

Cystine stones are produced in patients with a homozygous recessive gene for cystine transport. This causes abnormal transport of aminoacid in the kidney and gastrointestinal system, leading to build up of cystine. Cystine is an amino acid of cysteine-S-S cysteine. Normal individuals generally excrete into
urine less than 100 mg cystine/day whereas the majority of homozygous cytinurics excrete more than 200mg/day. There are no known inhibitors of cystine (Becker, 2007).

Figure 3
Types of stones

2.3.5 Xanthine stones

These types of stones are composed of xanthine, a nitrogen compound. These are extremely uncommon and usually occur due to rare genetic disorder known as xanthinuria or xanthine oxidase deficiency. It is caused by the deficiency of enzyme xanthine oxidase. In some cases, xanthine stones develop in patients being treated with allopurinol for gout (Arikyants et al., 2007).
2.4 Inhibitors of stone formation

Various substances in the body have an effect on one or more of the above stone forming processes, thereby influencing a person’s ability to promote or prevent stone formation. Promoters of stone formation facilitate stone formation while inhibitors prevent it (Jawalekar et al., 2010). Inhibitors reduce the rate of growth or aggregation of crystals or reduce adherence of crystals to the renal epithelium. Many inorganic and organic substances, high urine volume are known to inhibit crystal growth, aggregation and adhesion (Gupta et al., 2011b)

2.4.1 Inorganic inhibitors

2.4.1.1 Citrate

Citrate obtained through both endogenous and exogenous source is a tricarboxalic acid that circulates in blood. It causes increase in pH that result in increased citrate excretion. This effects the calcium- citrate- phosphate complex formation where the alkalization increases the solubility of uric acid that prevents salting out of calcium oxalate by urates. It prevents calcium supersaturation in the urine by forming complexes with calcium. It also increases stone crystallization inhibition property of other macromolecules. (Grases et al., 2006b)

2.4.1.2 Magnesium

Magnesium is mostly found in the bones and is the fourth abundant mineral in the body. Magnesium can form complexes with oxalate and reduces the risk of supersaturation. Magnesium supplementation to patients with magnesium deficiency increases the excretion of citrate in the urine thereby, reducing the risk of precipitation of stone forming constituents (Reungjui et al., 2002)

2.4.1.3 Pyrophosphates

Pyrophosphate is a potent inhibitor of crystal growth and in some cases also of crystal aggregation. Pyrophosphate and diphosphate have shown to inhibit the precipitation of calcium phosphate, where as diphosphates also inhibits the growth of apatite crystals. Pyrophosphate will reduce the absorption of calcium in the intestine (Basavaraj et al., 2007).
2.4.2 Organic inhibitors

2.4.2.1 Osteopontin (Uropontin)

This is a negatively-charged aspartic acid rich protein that inhibits growth of calcium oxalate crystals in a supersaturated solution. It is synthesized within the kidney and present in the human urine at levels in excess of 100 nM. It is an abundant component of organic matrix of calcium oxalate stones. *In vitro* studies suggest that osteopontin may inhibit nucleation, growth and aggregation of calcium oxalate crystals and also inhibits the crystal adhesion to renal epithelial cells. Some studies have reported decreased concentrations of osteopontin in urine of stone formers as compared to normal individuals (Mohamaden et al., 2014). It may direct CaOx crystallisation to the CaOx dihydrate (COD) phase rather than the CaOx monohydrate (COM) phase, the dehydrate being less adherent to renal tubular epithelial cells (Wesson et al., 2003).

2.4.2.2 Urinary prothrombin factor I (UPTF I)

This is a potent inhibitor of calcium oxalate stone formation *in vitro* (Chutipongtanate et al., 2005). The organic matrix of calcium oxalate crystals contains UPTF I, providing evidence that links the role of blood coagulation proteins with stone formation. UPTF I is an important inhibitor of calcium oxalate crystal growth, aggregation and adherence of crystals to renal cells (Webber et al., 2006).

2.4.2.3 Tamm- Horsfall Protein (THP)

Tamm and Horsfall isolated a mucoprotein from the human urine nearly 50 years ago. Tamm-Horsfall protein (THP), also known as uromucoid, is an 80-kDa glycoprotein synthesized exclusively in the thick ascending limb of the loop of Henle’s loop. THP is the most abundant protein in the urine of normal mammals. Much controversy exists about whether THP is a promoter or an inhibitor of crystal aggregation. Most authors believe that it is an effective inhibitor of COM crystal aggregation in solutions with high pH, low ionic strength and low concentration of divalent ions and THP. In contrast, with low pH, high concentrations of calcium, sodium, and hydrogen ions as well as low THP,
inhibitory activity is lost and it may even become a promoter of aggregation (Jaggi et al., 2007; Aggarwal et al., 2013). Self-aggregation of THP may promote either heterogeneous nucleation or formation of a protein and crystalline mass large enough to block the tubular lumen (Mo et al., 2004)

2.4.2.4 Glycosaminoglycans (GAGs)

GAGs are enzymatic products of proteoglycans and have been identified as one of the macromolecules present in the stone matrix. They are believed to play an important role in calcium oxalate crystallization. They have the ability to inhibit the growth and aggregation of calcium oxalate crystals by blocking the growth sites. They also prevent crystal adhesion to renal cells, which is an important step in renal stone formation. However, no study shows qualitative or quantitative significant difference in total excretion of glycosaminoglycans between stone formers and non-stone formers (Watts, 2005; Ou et al., 2015)

2.4.2.5 Renal lithostathine

Renal Lithostathine, a protein of pancreatic secretion, is an urinary inhibitor of calcium carbonate crystal growth. Several reports showing the presence of calcium carbonate (CaCO$_3$) in renal stones suggested that crystals of CaCO$_3$ might be present in the early steps of stone formation. Such crystals might therefore promote CaOx crystallization from supersaturated urine by providing an appropriate substrate for heterogeneous nucleation (Grover et al., 2002)

2.4.3 High urine volume

One of the most important inhibitors of stone formation is high urine volume. Increased urine volume achieved by a high fluid intake exerts an efficacious preventive effect on the onset and recurrence of urinary stones. A high water intake and urine dilution results in a marked reduction in saturation of lithogenous salts. The type of fluids should be carefully selected to achieve the appropriate change of urine composition depending on stone composition. A sufficient intake of fluid is one of the most important preventive measures for stone recurrence (Parks et al., 2003; Siener and Hess, 2003).
2.5 Promoters of lithiasis

Urine contains substances that influence crystallization processes, and therefore regulate stone formation. Substances that increase crystallization are termed promoters. Low urine volume, low urine pH, calcium, sodium, oxalate, and urate are known to promote stone formation. On the cell surfaces of the kidney, cell debris, protein aggregates and other crystals may provide analogous site for nucleation. These nucleation sites may lower the supersaturation required to initiate crystallization and therefore promote CaOx crystallization. Strong geometric similarities between the crystals of uric acid dihydrate and COM may promote overgrowth of one on the other, a process similar to the relationship between apatite and COM (Lonsdale, 1968; Chibber et al., 2014). Another factor that may promote the formation and growth of intra-renal crystals is ionic calcium. Hypercalciurria can decrease inhibitor function and lead to crystallization. Furthermore, cellular responses to newly formed crystals and factors that modulate these crystal-cell interactions could stimulate the initiation of an intra-renal stone. The role of cell injury may be an even more important determinant in the promotion and progression of kidney stones (Miller and Lingeman, 2007).

2.6 Mechanism of formation of renal stones

The formation of renal stones is a consequence of increased urinary supersaturation with subsequent formation of crystalline particles. Supersaturation is the driving force for crystallization in solutions like urine. If inhibitors of crystallization were not able to act, the final result will be nephrolithiasis. The point at which saturation of a solution is reached, and crystallization begins is commonly known as thermodynamic solubility product. Inhibitors allow higher concentration of calcium salts to be held in solution than in pure solvents. Urine is thus metastable with respect to calcium salts (Pandeya et al., 2010).

Calcium stone formation involves different phases of increasing accumulation of CaOx and Calcium Phosphate (CaP) that include nucleation, crystal growth, crystal aggregation and crystal retention.
2.6.1 Crystal nucleation

The initial step in crystallization of the supersaturated solution is called nucleation. This process begins with the combination of stone salts in solution into loose clusters called the nuclei or nidus. These nuclei may increase in size by addition of new components or clusters (Parmar, 2004).

Two types of nucleation occur homogenous and heterogeneous. Homogeneous nucleation occurs when calcium and oxalate ions complex to form small crystals which then grow to larger pure calcium oxalate stones. Heterogeneous nucleation occurs when calcium and phosphate ions or uric acid stones initially form small crystals of calcium phosphate. These small crystals form the substrate upon which calcium oxalate subsequently deposits. This process is known as “Epitaxy” where the crystals have similar lattice dimension (De Yoreo et al., 2006). Once a nucleus is created and is anchored, crystallization can occur at lower chemical pressures than required for the formation of the initial nucleus. Renal tubular cell injury supports crystallization of CaOx crystals by providing substances for growth and aggregation. Membrane vesicles produced by renal injury are good nucleators of calcium crystals (Evan et al., 2007).

2.6.2 Crystal growth

Crystal growth is one of the prerequisites for particle formation. After nucleation, crystal growth is the next major step in renal stone formation. The driving force behind this is the reduction in the potential energy of the atoms or molecules when they bond to each other. Crystal growth is determined by the molecular size and shape of the molecule, the physical properties of the material, supersaturation levels, pH, and defects that may form in the crystal’s structure (Aggarwal, 2010b).

2.6.3 Crystal aggregation

In this process, crystals in solution stick together and form a larger particle. Aggregation of particles in solution is determined by a balance of forces,
which may determine the aggregation or disaggregation of the stones. A small inter particle distance increases attractive force and favours particle aggregation. In addition, Tamm-Horsfall glycoprotein and other molecule may act as glue and increase viscous binding. In various steps of stone formation, crystal aggregation is a more important factor than nucleation and growth because aggregation occurs within seconds (Patel et al., 2010a).

### 2.6.4 Crystal retention

Urolithiasis requires formation of crystals followed by their retention and accumulation in the kidney. Crystal retention can be caused by the association of crystals with the epithelial cells lining the renal tubules. Crystal formation predominantly depends on the composition of the tubular fluid; crystal retention might depend on the composition of the renal tubular epithelial cell surface. The process of attachment or endocytosis of crystals to renal tubular cells is generally meant by crystal-cell interactions. These structural and functional studies of crystal-cell interactions in culture indicate that COM crystals rapidly adhere to microvilli on the cell surface and are subsequently internalized (Aggarwal et al., 2013).

### 2.7 Diagnosis of kidney stones

The patient with a kidney stone suddenly develops pain in the side or back, just below the ribs. This pain is known as renal colic. The pain may travel down towards the groin and may be very severe. It may be accompanied by feeling very unwell, loss of appetite, vomiting and blood in the urine. The diagnosis of a kidney stone can be made by symptoms alone. Once the diagnosis is made, a decision of whether to treat the stone or let it pass will be made based on the stone’s size, location and the level of pain. The first step in diagnosis is ruling out the possibility of other diseases causing similar symptoms (Carter and Green, 2011).
2.7.1 Laboratory tests

Urine analysis should be performed in all patients with suspected calculi. Aside from the typical microhematuria, important findings to note are the urine pH and the presence of crystals, which may help to identify the stone composition. Patients with uric acid stones usually present with an acidic urine, and those with stone formation resulting from infection have an alkaline urine. It is also tested for
the presence of chemicals that inhibit or promote stone formation. The urine may also be tested (cultured) for the presence of infection-causing organisms. A 24-hour urine collection may be needed to measure urine volume and levels of acidity, calcium, sodium, uric acid, oxalate, citrate, and creatinine. Urine tests that are used to determine the specific chemical and biological factors causing the stone should be performed about 6 weeks after the attack, since the attack itself may change the levels of such substances, including calcium, phosphate, and citrate (Portis and Sundaram, 2001). A blood sample must be obtained for serum creatinine, calcium (total or ionized), phosphate and uric acid determinations (Vezzoli et al., 2005)

2.7.2 X-Ray

Standard X-rays of the mid to lower abdomen will include the kidneys, ureter and bladder. X-rays work by passing low doses of radiation through the patient's body onto a photographic negative. When X-rays pass through soft body tissues, they hit the film and cause it to turn black (exposure). When a calcium stone or bone (which is also made of calcium) is present, the X-rays cannot pass through and no exposure occurs. The stone(s) and bones will appear white. This technique can only detect stones which contain calcium. It will miss pure uric acid stones. X-rays can be done quickly and cheaply and are a quick, inexpensive, and useful technique for monitoring growth of a kidney stone (Wells et al., 2012)

2.7.3 Ultrasound

Ultrasound is performed by passing a probe over the kidneys, ureters, and bladder. Sound waves are emitted from the probe and are integrated into an image of the urinary system that can be seen on a television screen. This test can detect both calcium and non-calcium types of stones. It is not a good test to find a stone that is passing from the kidney through the ureter on its way to the bladder. This is the time when stones hurt the most and cause people to seek immediate emergency medical assistance. If the stone has been lodged in the
ureter for some time, then it can detect obstruction. In this situation, the ureter is abnormally dilated. This technique is used for routine monitoring of new stones or growth of old stones. It has the benefit of no radiation (Patlas et al., 2001).

2.7.4 Computerized Tomography (CT scan)

This is one of the best methods to detect kidney stones, especially when someone comes to the emergency room with severe pain (colic) due to a passing stone. It is more sensitive than ultrasound or X-ray. It is performed by placing the patient in an X-ray tube that creates several images of the kidneys, ureter, and bladder. It can detect both calcium and non-calcium type of stones. It is more expensive than an X-ray and requires more radiation. Since it scans many organs and it can even sometimes detect non-stone causes of severe pain (Shokeir et al., 2004).

2.7.5 Intravenous Pyelogram (IVP)

This is one of the older techniques for detecting kidney stone and is still in use. A special dye is injected into a vein. Then X-rays are taken from mid to lower abdomen. If a stone is present, a filling defect will be seen on the X-ray images. It is very useful for detecting stones in the ureter, especially if not seen by CT scan. This sometimes happens when the ureter is dilated/obstructed but no stone is not detected. One disadvantage is that the injected dye can cause allergic reactions, usually temporary kidney damage, and symptoms including nausea, if the patient has kidney disease other than stones, sometimes it is better to perform a retrograde urogram. In this test, a catheter is placed into the bladder and the dye is injected to visualize the bladder and ureters for stones. This technique avoids absorption of the dye outside of the urinary tract (Pfister et al., 2003).

2.7.6 CT Urography

CT urography is a combination of CT and IVP. An injection of intravenous dye is given which outlines the parts of the kidney, ureter, and bladder where urine collects. The images are viewed with a CT scanner. Traditional CT images are also generated. This test is particularly helpful as a step in the evaluation for
blood in the urine (hematuria). It is particularly useful in the evaluation of a kidney (renal) diverticulum, a pouch that develops inside the kidney. Kidney stones and infections can form inside this pouch. It can also be associated with pain (Richmond, 2007)

2.8 Management of stones

Most ureteral stones that are less than 5mm in diameter pass spontaneously, whereas stones that are 7mm or more have a poor chance of passing. The different methods of kidney stone management are discussed below.

2.8.1 Non invasive therapy

2.8.1.1 Fluid intake therapy

One of the non invasive method to control kidney stone is to increase the intake of fluids. People diagnosed with kidney stones should drink 64 ounces of water each day. Drinking plenty of fluids also reduces the risk of urinary tract infections—a major cause of struvite stones (Bijarnia et al., 2010).

2.8.1.2 Dietary changes

Patients with calcium oxalate or uric acid stones may need to reduce the amount of meat products and table salt in their diets and increase fibre. Minimizing the intake of dairy products and oxalate rich foods (Holoch and Tracy, 2011).

2.8.1.3 Drug therapy

Prescribing certain medications may help prevent calcium and uric acid stones. These medications control the amount of acid or alkali in the urine, major role player in the crystal formation.

The medicine allopurinol may also be useful in some cases of hyperuricosuria. This drug interferes with the production of uric acid in the liver, reducing heterogeneous nucleation of calcium oxalate by both uric acid and monosodium urate. In addition, the adsorption of normally occurring macromolecular inhibitors of calcium oxalate crystallization by uric acid or monosodium urate could be possibly averted when using this drug (Yasui et al., 2001).
Hypercalciuria can be controlled by use of certain diuretics, such as hydrochlorothiazide. Thiazide lowers urine calcium resulting in a fall in calcium oxalate and calcium phosphate supersaturation. These diuretics act by increasing calcium absorption in proximal tubule and early distal convoluted tubule (Nijenhuis et al., 2005).

Potassium citrate reduces urinary saturation of calcium salts by complexing calcium and reducing ionic calcium concentration. Due to its alkalinizing effect, it also increases the dissociation of uric acid, lowers the amount of poorly soluble undissociated uric acid, reducing the propensity to form uric acid stones. The induced decline of urinary calcium during the early period of treatment represents a potential additional advantage of the drug. Therefore, potassium citrate seems to be effective in conditions of hypocitraturia, hypercalciuria, or hyperuricosuria (Shekarriz and Stoller, 2002).

Rarely patients with hypercalciuria are given the medicine sodium cellulose phosphate that bind to calcium in the intestine and prevents leakage into the urine. Thiola and cuprimine may be given for the treatment of cystine stones, potassium forms of alkali are preferred to decrease uric acid and increase urate concentration as a treatment for uric acid stones. Beside these, antibiotics, a urease inhibitors are used for the treatment of stones with infections. If struvite stone cannot be removed, acetohydroxamic acid is given, this has been used with long term antibiotics to prevent infections that lead to stone formation (Lake and Brown, 1985; Sakhaee et al., 2012).

2.8.2 Invasive therapy

2.8.2.1 Extracorporeal shock wave lithotripsy (ESWL)

ESWL is a completely non-invasive form of treatment, the most frequently used procedure for eliminating kidney stones. In most cases, shock wave lithotripsy is done on an outpatient basis. Recovery time is short and most people can resume normal activities in a few days. Shock wave treatment uses a machine called a lithotripter. It works by directing ultrasonic or shock waves, created outside the body ("extracorporeal") through skin and tissue, until they hit
the dense kidney stones. The impact causes stress on the stone. Repeated shock waves cause more stress, until the stone eventually crumbles into small pieces. These sand-like particles are easily passed through the urinary tract in the urine. The technology is only effective if the kidney is functioning well and there is no blockage to the passage of stone fragments (Srinivas et al., 2012).

Figure 6

Extracorporeal shock wave lithotripsy (ESWL)

While shock wave lithotripsy is considered safe and effective, it can still cause complications. Most patients have blood in their urine for a few days after treatment. Bruising and minor discomfort in the back or abdomen from the shock waves are also common. To reduce the risk of complications, urologists always suggest patients to avoid aspirin and other drugs that affect blood clotting for several weeks before treatment. Another complication may occur if the shattered stone particles cause discomfort as they pass through the urinary tract. In some cases, the urologist will insert a small tube called a stent through the bladder into the ureter to help the fragments pass (Evan and Willis, 2007).

2.8.2.2 Uteroscopy

This treatment involves the use of a very small, fiber-optic instrument called an ureteroscope, which allows access to stones in the ureter or kidney. The ureteroscope allows the urologist to directly visualize the stone by progressing up the ureter via the bladder. No incisions are necessary and
general anesthesia is used to keep the patient comfortable during the procedure. Once the stone is seen through the ureteroscope, a small, basket-like device can be used to grasp smaller stones and remove them. If a stone is too large to remove in one piece, it can be fragmented into smaller pieces. Most commonly this is accomplished with laser energy (Fine et al., 2010).

**Figure 7**

**Uteroscopy**

2.8.2.3 Percutaneous nephrolithotomy (PNL)

PNL is the treatment of choice for large stones located within the kidney that cannot be effectively treated with either ESWL or URS. General anesthesia is required to perform a PNL. The main advantage of this approach compared to traditional open surgery is that only a small incision (about one centimeter) is required in the flank. The urologist then places a guide wire through the incision. The wire is inserted into the kidney under radiographic guidance and directed down the ureter. A passage is then created around the wire using dilators to provide access into the kidney. An instrument called a nephroscope is then passed into the kidney to visualize the stone. Fragmentation can then be done using an ultrasonic probe or laser. Because the tract allows passage of larger instruments, the urologist can suction out or grasp the stone fragments as they are produced. This results in a higher clearance of stone fragments than with ESWL or URS (Asplin et al., 2003).
2.8.2.4 Open surgery

A large incision is required in order to expose the kidney or portion of ureter that is involved with the stone. The portion of kidney overlying the stone or the ureteral wall is then surgically cut and the stones are then removed (Healy and Ogan, 2007).

The high cost and adverse effects of minimally invasive techniques, and lack of satisfactory drug to use in clinical therapy, especially for the prevention or the recurrence of stones, alternative treatment modalities with phytotherapeutic agents have become the mainstay of medical therapy (Rathod et al., 2012).

2.9 Medicinal plants

Since the dawn of human civilization, men have used plants as a source of medicine, because they are easily available in the immediate environment. Ancient medicine was not solely based on empiricism and this is evident from the fact that some medicinal plants which were used in ancient times still have their place in modern therapy (Mondal et al., 2013). Nature has provided an excellent storehouse of remedies to cure all kinds of ailments. Ayurveda is recorded in ancient scriptures handed down through generations and developed over 6000 years.
This time-tested holistic medicinal system maintains that good health exists when the body, mind, spirit and environment are in perfect harmony. Good health is a rare occurrence in this age where people live in stressful environment and follow unplanned diet and unbalanced life style. It is the need of the hour that a new vibrant medical system evolved which is devoid of side effects and leads to the revival of ayurvedic traditions. Herbal and herbal based medicines are expected to form basis for such development. Even in the era of genetic engineering, plants account for forty percent of all medicinal formulations prescribed (Dasture, 2002).

The global awareness of everything natural is the biggest challenge for Indian pharmacist to come out with newer technologies (Divakar, 2002). Herbal drugs are now being developed in dosage forms using modern manufacturing and processing techniques. Modern herbal research is focused mainly on activity-guided isolation of phyto-constituents from crude drug. Herbal medicines contain compounds whose effects can be demonstrated pharmacologically and the action of whole plant extract can usually be related to the isolated constituents (Jain and Venkatasubramanian, 2014)

When compared to other methods of drug development, there are several reasons why naturally produced compounds could make better therapeutic candidates. First and foremost, it reduces the strenuous process of manufacturing new drugs to improve already existing drugs. Herbal formulations are useful as the physiological effects of different botanical species have already been discovered. A simple look into our history could inspire drug developers into the next step of drug research for specific diseases. Another advantage that natural compounds have over synthetic drug development is that there is often more data on the long term effects of the treatments as we may already have the long term effects documented (Cazonelli, 2011).

**2.9.1 Phytochemicals and Antioxidants**

Phytochemicals are compounds that are produced by a wide variety of plants and have an effect on human health. Phytochemicals are found in plant-based foods such as fruits, vegetables, beans, and grains. There is some
evidence that a diet rich in fruits, vegetables, and whole grains reduces the risk of lifestyle diseases. Researchers are looking for specific compounds in these foods that may account for these healthful effects in humans (Kushi et al., 2012).

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Vasu et al., 2009). These compounds have been synthesized by plants as a biochemical defences to cope with the constraint of their sessile existence. When an animal is attacked, it can fight or run away, and when a plant is attacked it fights back too, just on a whole new level. The type of molecules most often employed by plants in their defences is called secondary metabolites, because they are made using supplementary reactions, which are usually the continuation of a pathway. The chemical compounds generated from the complex metabolic reactions can also be further modified into adapted variations, often unique to species. These chemical compounds are phytochemicals that can act like a hormone or antioxidant (Taiz and Zeiger, 2010).

Antioxidants are compounds that act as inhibitors of the oxidative process. They are quite large in number and diverse, which oppose the process of oxidation largely by neutralizing free radicals. The molecules at relatively small concentrations have the ability to inhibit the oxidant chain reaction. These are of paramount importance to pharmaceutical formulations because there are innumerable medicinal agents possessing diverse chemical formulations and are known to undergo oxidative decomposition (Asaduzzaman et al., 2013). The activity of natural antioxidants is due to the presence of substituted groups such as carboxyl group, electron withdrawing group, electron donating group etc which may be phenolic or non-phenolic (Kshirsagar and Upadhyay, 2009).

2.10. Medicinal plants selected in the study

Man tries to grow the sort of plants that he wants and the original inhabitants of the soil become useless to him are called as weeds. Weeds are not desired in arable lands since they compete with crop plants for nutrients, soil
moisture, sunlight and space. They have often been given special identity as a fast growing troublesome exotic and noxious plant, in other words known as unwanted plants growing among the normal seasonal crops. Unlike other crop plants, weed plants are less vulnerable to disease and insect attack (Dhanam, 2014).

**Characteristics of weeds**

Weeds are also like other plants but have special characteristics that tend to put them in the category of unwanted plants.

- Most of the weeds produce enormous quantity of seeds
- Weeds have the capacity to withstand adverse conditions in the field, because they can modify their seed production and growth according to the availability of moisture and temperature. They can germinate under adverse soil-moisture conditions, have short period of plant growth, generally grow faster rate and produce seed earlier than most of the crops growing in association.
- Weed seeds remain viable for longer period without losing their viability,
- Weed seeds have a tremendous capacity to disperse from one place to another through wind, water and animals including man. Many of times, weed seeds mimic with the crop seeds due to their size and get transported from one place to another along with them (Sankar and Satapathy, 2015).

There is a renewed interest in focusing on utilization of weeds in productive ways, so that people may benefit from an aspect that has been largely ignored. ‘Utilization’ has been recognized as an effective means of weed management. Tribal and some of the rural people make great use of spontaneous plants. The wild plants make an important contribution, particularly to the diet of the local inhabitants, apart from other useful products. Weeds are highly valued in traditional medicine and the pertinent traditional knowledge was transferred orally through generations (Chakraborty and and Duary, 2014).
Weeds can be useful to us if we learn to use them, weeds can be utilized as manure, fodder, livestock beds, dye and as therapeutic agents because of the presence of some chemicals like glycosides and alkaloids. They can be used as biopesticides and are highly efficacious as medicine against common diseases like cold, asthma and dermatitis and other health problems of man (Patel et al., 2014).

2.10.1. *Tribulus terrestris*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
<td>Zygophyllales</td>
</tr>
<tr>
<td>Family</td>
<td>Zygophyllaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Tribulus</td>
</tr>
<tr>
<td>Species</td>
<td><em>T. terrestris</em></td>
</tr>
</tbody>
</table>

*Tribulus terrestris* is a flowering plant belonging to the family Zygophyllaceae, native to tropical region. It is commonly known in Tamil as Siru Nerunji. It is a herbal remedy used for various purposes in folk medicine. It has been used as tonic, aphrodisiac, astringent, analgesic, diuretic and urinary anti-septic agents (Kor et al., 2013).

2.10.2. *Aerva lanata*

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Order</td>
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</tr>
<tr>
<td>Family</td>
<td>Amaranthaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Aerva</td>
</tr>
<tr>
<td>Species</td>
<td><em>A. lanata</em></td>
</tr>
</tbody>
</table>

*Aerva lanata* belongs to the family Amaranthaceae. It is commonly known as Sirupeelar in Tamil or Siddha (Khare, 2007). *A. lanata* is an herbaceous perennial weed (Manokaran et al., 2008). It is indigenous in India, Srilanka, South Asia, Saudi Arabia, tropical Africa and South Africa. It is used as an important
medicinal plant for illness. In English, it is called as a stone breaking plant. *A. lanata* comprises medicinal and pharmaceutical importance. In the Traditional System of Medicine, the plant is being used as diuretic and anthelmintic, antidiabetic and treatment of lithiasis (Rajesh *et al.*, 2011). It possesses a variety of medicinal properties like hepatoprotective and hyperglycemic activity (Ragavendran *et al.*, 2012).

### 2.10.3. *Scoparia dulcis*

- **Kingdom**: Plantae
- **Order**: Lamiales
- **Family**: Plantaginaceae
- **Genus**: *Scoparia*
- **Species**: *S. dulcis*

*Scoparia dulcis* Linn. is an erect annual herb also known as Kalluruki belongs to the family *Scrophulariaceae* (Dicot). It grows as wasteland herb and has many promising traditional use in medicine (Saikia *et al.*, 2011). It is a perennial herb widely distributed in tropical and subtropical regions. The fresh and dry plants have traditionally been used as one of remedies for stomach troubles, hypertension, diabetes, inflammation, bronchitis, hemorrhoids and as an analgesic and antipyretic (Mishra *et al.*, 2013).

### 2.10.4. *Tridax procumbens*

- **Kingdom**: Plantae
- **Order**: Asterales
- **Family**: Asteraceae
- **Genus**: *Tridax*
- **Species**: *T. procumbens*
Tridax procumbens is a common medicinal herb belonging to the family Asteraceae. It is a wide spread weed and pest plant. In Tamil Nadu, it is known as Vettu kai thalai. It has been found to possess significant medicinal properties. It is commonly used in Indian traditional medicine as anticoagulant, antifungal and insect repellant, in bronchial catarrh, diarrhea and dysentery. Moreover it possesses wound healing activity and promotes hair growth (Kuldeep and Pathak, 2013)

The layout of the study, the materials used and the methodology adopted are explained, with appropriate references quoted, in the following chapters.