INTRODUCTION
Biomineralization, which refers to the complex processes by which organisms form minerals, is frequently associated with a high degree of regulation on different hierarchical levels. “Calcium stone formation” is mineral crystallization in body tissue or fluid. The deposition of inorganic minerals, crystalline or noncrystalline, around biomolecules is universal in biology, where inorganic crystals are harnessed to become an integral part of organic tissue to provide hardness and strength. More than 200 yr ago, Hunter articulated about the similarity between stone formation and calcification pointing the equivalence of enamel, eggshell, gallstones, and kidney stones (Hunter, 1771). Thus, mineralization can be arbitrarily divided into physiologic and pathologic. Physiologic crystallization includes formation of exoskeleton, pearl, endoskeleton, and dentition, whereas pathophysiologic crystallization includes pyrophosphate arthropathy, pigmented gallstones, vascular calcification, and urolithiasis. Pathologic calcium crystallization is a physiologic process in the wrong place and the wrong time (Moe and Bonny, 2005). The majority of normal and pathological bio-minerals formed in humans are sparingly soluble electrolytes with basic anions (i.e. anions that can be protonated), such as phosphates, carbonates, oxalates, urates, etc. Their solubility thus, depends (often strongly) on pH (Königsberger and Königsberger, 2006).

Urolithiasis, i.e. the formation of stones or calculi in the urinary tract, is not only a painful condition affecting some 2 – 20% of the population worldwide but is also associated with high cost to the society because of the high prevalence of the disease and high recurrence rates (Johri et al., 2010; Lotan, 2009). The term urolith is derived from the Greek ouron meaning urine, and lithos meaning stone (Osborne et al., 1999). The oldest urolith of human origin was found in the Egyptian tomb of a 16-year-old
bov and dates from around 4800 B.C. (Ellis, 1979). Reference to stone formation is made in the early Sanskrit documents in India between 3000 and 2000 B.C. (Prasad et al., 2007). As it is clear from these historical clues, urinary stone has always been a common disease and presently it is the third most common affliction of the urinary tract (Atmani, 2003). Depending on the socio-economic conditions and subsequent changes in the dietary habits, the overall probability of stone formers differs in various part of world: 1-5% Asia, 5-9% Europe, 13-15% USA and 20% Saudi Arabia. The “stone belts” of the world are located in the countries of the Middle East, North Africa, Mediterranean regions, North-western states of India and Southern states of USA (Lopez and Hoppe, 2010). In India, with a prevalence rate of 15%, two high incidence stone belts have been found to occur. The first belt starts from Amritsar in North and while passing through Delhi and Agra ends up in U.P. The other belt which starts from Jamnagar in west coast extends inwards towards Jabalpur in central India. Very low incidence areas have been in West Bengal and coastal areas of Maharashtra, Karnataka, Kerala, Tamil Nadu, Andhra Pradesh (Rizvi et al., 2002; Tandon et al., 1999). The incidence of urinary stones has been increasing over the last few years while the age of onset is decreasing (Devuyst and Pirson, 2007). With the prevalence rate of >10% and an expected recurrence rate of ~50%, stone disease has an important effect on health care system (Knotl, 2007). For decades, urolithiasis has arguably been one of the most research intensive sectors of clinical and fundamental investigations into the cause, prevention and treatment of crystal deposition diseases in humans. However, it appears that a real breakthrough in this area is lacking as yet.

The formation of uroliths involves multiple physiological and pathological processes. Human body fluids are normally supersaturated with regard to several substances (e.g.
blood plasma, interstitial and intracellular liquors with respect to calcium carbonates and phosphates, particularly hydroxyapatite and fluoroapatite; bile with respect to cholesterol; urine with respect to calcium oxalates and, depending on the pH, with regard to uric acid or calcium phosphates). The question, why crystalluria is common but stone formation is not, has been discussed in terms of three main factors:

(i) the supersaturation as a necessary condition,

(ii) the presence of heterogeneous nucleants and

(iii) deficit of crystallization inhibitors (Grases and Costa-Dauza, 1999)

In a comprehensive review, different types of renal stones have been classified (Grases et al., 1998). These stones or calculi are majorly composed of calcium oxalate hydrates (calcium oxalate monohydrate and calcium oxalate dihydrate), ammonium magnesium phosphate (struvite), calcium phosphates (hydroxyapatite and brushite), uric acid and urates, cystine and xanthine. Figure 1.1 depicts various types of stones majorly found in human population alone or in combination.

The etiology of this disorder is multifactorial and is strongly related to dietary, lifestyle habits and climatic changes (Lopez and Hoppe, 2010). Management of stone disease depends on the size of calculi, severity of symptoms, degree of obstruction, kidney function, location of the stones and the presence or absence of associated infection influence the choice of one type of intervention over the other and ranges from observation (watchful waiting) to surgical removal of the stone.
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<tr>
<td>Calcium oxalate monohydrate</td>
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<td>Ammonium Acid Urate</td>
<td>Cystine</td>
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<td>Xanthine</td>
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Figure 1.1: Kidney Stones
Stones larger than 5mm fail to pass through and require assistance medically (Butterweck and Khan, 2009). Currently this serious problem can be treated with increased fluid intake, medications like thiazides, allopurinol (Atmani, 2003) and potassium citrate (Tracy and Pearle, 2009) for reducing urinary saturation of stone forming calcium salts and thus preventing nucleation, crystallization and agglomeration of calcium salts, diuretics for facilitating urine flow, surgical procedures such as extracorporeal shock wave lithotripsy (ESWL), endourological procedures such as ureterorenoscopy (URS) or percutaneous nephrolithotomy (PCNL) and the combination of these techniques (Gurocak and Kupeli, 2006).

Despite major technical advancements in treating kidney stones, the problem still persists. Treatments available till date have serious side effects and do not eliminate the probability of recurrence completely. Traumatizing effects of shock waves, persistent residual stone fragments after ESWL and a possibility of infection pose serious problems to be taken into consideration (Atmani, 2003). ESWL is also reported to be associated with effects as renal damage and hypertension (Butterweck and Khan, 2009). Furthermore, ESWL is known to be related to long term medical effects such as diabetes mellitus (Krambeck et al., 2006). Even the medications like thiazides cause intracellular acidosis and can lead to hypokalemia and hypocitraturia (Heilberg and Schon, 2006).

Actually, there are no satisfactory drugs in modern medicine, which can dissolve stone and the physicians remain to be dependent on alternative systems of medicine for better relief. Herbal medicines are efficacious and have lesser side effects compared to modern medicines and also reduce the recurrence rate of renal stone (Prasad et al., 2007). The complete mechanism of action of these remedies is lacking.
Unlike allopathic medicines which majorly target only one aspect of urolithiatic pathophysiology, most of the plant based therapies have been shown to be effective at different stages of stone pathophysiology. Currently known extracts exert their antilithogenic properties by multiple mechanisms like:

- Help in spontaneous passage of calculi by increasing urine volume, pH and anti-calcifying activity (Diuretic activity)
- Balance the inhibitor and promoters of crystallization in urine and affects the crystal nucleation, aggregation and growth (Crystallization inhibition activity)
- Relieve the binding mucin of calculi (Lithotriptic activity)
- Improve renal function
- Regulate oxalate metabolism
- Regulate the crystalloid colloid imbalance and improve renal function, thus prevents recurrence of urinary calculi
- Improve renal tissue antioxidant status and cell membrane integrity and prevents recurrence (Antioxidant activity)
- Exert significant anti-infective action against the major causative organisms (Antimicrobial activity)
- Relieve pain, burning micturition and haematuria (Analgesic and anti-inflammatory activity) (Pareta et al., 2011)

The marketed composite herbal formulations, Cystone (Himalaya Drug Company, India), Neeri (Aimil Pharmaceuticals, India), Uritone (Aimil Pharmaceuticals, India), Uriflow (Bioneutrix Labs), Culdisol (Ganga Pharmaceuticals, India), Calcury (Charak Pharmaceuticals, India), Chandraprabhabati (Baidyanath, India) and Culin Forte (Alopa Herbal) have been used worldwide to dissolve urinary calculi in kidney and
urinary bladder. *Tribulus terrestris* has long been an important constituent in tonics in Indian Ayurveda practice, where it is known by its Sanskrit name, “gokshura”. This is a common constituent of antiurolithiatic herbal formulations like Cystone, Neeri, Uritone, Uriflow, Culdisol and Culin forte.

*Tribulus terrestris* (Puncture Vine, Caltrop, Yellow Vine and Gokhru) is a flowering plant of the Zygophyllaceae family, native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, Africa and Northern Australia. It can thrive even in desert climates and poor soil.

![Figure 1.2: Tribulus terrestris](image)

The roots and fruits are sweet, cooling, diuretic, aphrodisiac, emollient, appetiser, digestive, anthelmintic, expectorant, anodyne, anti-inflammatory, alterant, laxative, cardiotoxic, styptic, lithotriptic and tonic. They are useful in strangury, dysuria, vitiated conditions of vata and pitta, renal and vesical calculi, anorexia, dyspepsia, helminthiasis, spermatorrhoea, anaemia, scabies, ophthalmia and general weakness. The leaves are astringent, diuretic, aphrodisiac, depurative, anthelmintic and tonic. They are useful in gonorrhoea, inflammation, menorrhagia, strangury, leprosy, skin
diseases, verminosis and general weakness. The seeds are astringent, strengthening and are useful in epistaxis, haemorrhages and ulcerative stomatitis. The ash of the whole plant is good for external application in rheumatoid arthritis (Warrier et al., 1996). The diuretic properties of the plant are due to the large quantities of the nitrates present as well as the essential oil which occurs in the seeds (Nadkarni, 1993).

In a preliminary study, the diuretic effect of *Tribulus terrestris* and *Hygrophila spinosa* water extracts in albino rats was evaluated (Kumari and Iyer, 1967; Singh et al., 1991). The effect of an aqueous extract of *Tribulus terrestris* administered orally at a dose of 5 g/kg body wt and restoration in urinary oxalate was observed (Sangeeta et al., 1993). Few studies were conducted to evaluate the therapeutic use of *Tribulus terrestris* in various urinary disorders including urolithiasis (Anand et al., 1994; Sangeeta et al., 1994). The inhibitory potency of the extract of putatively litholytic plant, *Tribulus terrestris* was tested on the growth of brushite and CaOx crystals in vitro and it exhibited appreciable amount of inhibition (Joshi et al., 2005a; 2005b).

As discussed above, various marketed antiurolithiatic herbal formulations like Cystone (Himalaya Drug Company), Neeri (Aimil Pharmaceuticals), Uritone (Aimil Pharmaceuticals), Uriflow (Bioneutrix), Culdisol (Ganga Pharmaceuticals) and Culin forte (Alopa Herbal) contain *Tribulus terrestris* as a common constituent in different proportions.
Keeping in view the importance of *Tribulus terrestris* as an antiurolithiatic agent and the complications arising due to the surgical treatment of kidney stones available, the study was designed to further investigate its antilithiatic potency *in vitro* and *in vivo* with the following objectives:

1. To study the effect of aqueous extract of *Tribulus terrestris* on calcium oxalate crystallization.

2. To study the effect of *Tribulus terrestris* on oxalate induced injury in rat renal epithelial cell lines (NRK-52E).

3. To investigate the prophylactic and curative role of aqueous extract of the *Tribulus terrestris* on experimentally induced nephrolithiatic rats.

4. To isolate, purify and characterize the new biologically active compounds (potent biomolecules) from *Tribulus terrestris* which have the ability to influence calcium oxalate crystal growth.