CHAPTER 1
INTRODUCTION

Among urinary tract disease, urolithiasis is the third most frequent urological afflictions affecting mankind for millennia [1]. Literature indicates that the occurrence of kidney stones dates back to pre-historic era. The oldest renal stone on record was described by Shattock in 1905 and was found in an Egyptian mummy in a tomb dating to approximately 4400 B.C. [2]. This 1.5 cm calciferous calculi lay beside the first lumbar vertebra. Stone disease affects 2-20% population worldwide with a prevalence rate of 15% in India [3, 4]. After passage of a first stone, the risk of recurrence is 40% at 5 years and 75% at 20 years [5]. The "stone belt regions" of the world are located in countries of Middle East, North Africa, the Mediterranean Regions, North Western state of India and Southern State of USA [6]. In India, two stone belt regions are observed. First, Amritsar in North, passing through Delhi and Agra ends up in UP. Second, Jamnagar in the west coast extends inwards towards Jabalpur in Central India [7]. Changes in socioeconomic conditions over time, and the subsequent changes in dietary habits, have affected not only the incidence but also the site and chemical composition of calculi. Stone composition has changed substantially over the past decades, with a progressive increase in frequency of calcium oxalate and calcium phosphate stones. Recent epidemiology studies from different continents and countries report that calcium oxalate accounts for 70% of stones, followed by calcium phosphate (~10%), struvite (~10%), uric acid (~5%), cystine (~1%), and mixed or miscellaneous (~4%) [6, 8].

It is a well-known fact that water is a pivotal element in digestion, circulation, elimination, and regulation of body temperature. A critical function of the urinary system is the maintenance of normal composition and volume of body fluid; this is accomplished by glomerular filtration, tubular reabsorption, and tubular secretion of soluble and filterable plasma components. As a result of this, urine contains water, electrolytes, minerals, hydrogen ions, electrically active ions, end products of protein metabolism, small and macromolecular organic components and other compounds not useful to the metabolism, energy requirements, or structure of the body [9]. Human urine is a complex solution containing not only calcium (Ca) and oxalate (Ox) but also
other ions and macromolecules that can interact with Ca and/or Ox and modulate crystallization. Under normal circumstances, urine will contain crystals but not stones. Some urinary constituents, referred to as inhibitors of crystal formation, enable the urine to retain more ions in solution than at the level of saturation [9].

In the study of urolithiasis, formation of CaOx kidney stones is the consequence of extracellular as well as cellular events. Extracellular events include supersaturation, crystal nucleation, growth and aggregation and occur in renal tubular lumens and renal pelvises. Cellular events occur in cells of the renal epithelium and interstitium. These include management of acid base balance, urinary citrate, oxalate, calcium, magnesium, and pH as well as response of renal epithelial cells to the changing urinary environment, particularly oxalate overload and the presence of calcium oxalate crystals. The interplay between renal epithelial cells and Ox and/or CaOx crystals alters renal cell functions, changes the extracellular environment and plays a significant role in the formation of CaOx stones [10].

The driving force for crystallization is the development of supersaturation with respect to the precipitating salt. Since most of the ions crystallizing within the urinary tract will be excreted freely, crystal formation is by no means equivalent to symptomatic stone disease. However, when crystals are retained within the kidney, they can grow to become full-size stones [11]. Crystals can be retained at many sites in the kidneys and undergo the size-enhancing process of growth and aggregation. In order for stones to be formed, not only do crystals need to be retained within the kidney, but they must be located at sites from which crystals can cause ulceration at the papillary surface to form a stone nidus. It is thought that renal tubular injury plays an important role at this point. Khan hypothesized that renal tubular injury promotes crystal retention and the development of a stone nidus on the renal papillary surface [12]. In addition, renal tubular injury enhances crystal nucleation at low supersaturation [13]. Crystal–cell interaction is the next step, and is also promoted by renal tubular injury. Since crystal formation is a common phenomenon in human urine and crystalluria per se is harmless, abnormal retention of formed particles must occur when kidney stones form. Thus, crystal–cell interactions may be highly relevant. The crystals that are internalized in the interstitium undergo growth and aggregation, and develop into renal stones.

Despite the major technical achievements for stone removal, the problem of recurrence persists. Management of stone disease depends on the size and location of the stones.
• The foremost treatment which is considered is with pain medication, as the worst pain known as colicky pain is produced in the lower back.
• The formed calculi are most commonly removed by surgical methods, but the rate of recurrence is high in this case.
• The ultrasonic energy is used to break and reduce the size of the stone to make them easily pass in urine, but this is not beneficial in all the cases as some larger stones do not respond to this energy.
• ESWL uses sound waves which are also known as shock waves to break the stones in to small pieces for their easy passage out.
• Many allopathic agents like thiazide diuretics (e.g. Hydrochlorothiazide), alkali (e.g. Potassium citrate), allopurinol, sodium cellulose phosphate (SCP), penicillamine (Cuprimine), analgesic (Diclophenac sodium), bisphosphonates, and potassium phosphate are prescribed for the management of kidney stones and their symptoms.
• *Oxalobacter Formigenes* and other probiotics are used in treating the stones formed which act by decreasing the excretion of stone forming agents such as oxalates, calcium, phosphates etc. [14].
• The ayurvedic medicine used in the treatment are Cystone, Calcuri, Chandraprabha bati, Trinapanchamool, Rencare Capsul, Patherina tablet, Ber Patthar Bhasma, Chander Prabha vati.

Development of modern techniques such as ESWL, PCNL, or URS have revolutionized surgical management of kidney stones in recent years but do not give satisfactory results as these techniques do not prevent the likelihood of new stone formation [15]. Various medicinal plants have been used since ages to treat urinary stones though the rationale behind their use is not well established through systematic and pharmacological studies, except for some composite herbal drugs and plants [16]. In recent years, numerous studies describing the therapeutic properties of extracts from different parts of various medicinal plants have been developed. Indeed, the use of such extracts as complementary and alternative medicine has lately increased, and also serves as an interesting source of drug candidates for the pharmaceutical industrial research [17].

*Terminalia arjuna* belonging to the family Combretaceae, is a deciduous tree found throughout India and it’s thick white to pinkish-grey bark has been used in India’s
native Ayurvedic system of medicine for over three centuries, primarily as a cardiac tonic [18] as shown in Figure 1.1. *Terminalia arjuna* bark helps maintain a healthy heart and reduces the effects of stress and nervousness (Himalaya herbal health care). It also promotes effective cardiac functioning and regulates blood pressure. Experimental and clinical studies revealed the beneficial effects of this plant against all sorts of conditions of cardiac failure [19], dropsy, antinfective [20], antiasthematic and for the treatment of rheumatoid arthritis. *Terminalia arjuna* therapy for two weeks leads to significant regression of the endothelial abnormality amongst smokers [21]. The bark of *Terminalia arjuna* is also reported to inhibit nitric oxide production in murine macrophages [22]. Aqueous extract of the bark of *Terminalia arjuna* is shown to protect the liver and kidney tissues against CCl₄-induced oxidative stress probably by increasing antioxidative defense activities [23]. Casuarinin extracted from *Terminalia arjuna* attenuates H₂O₂-induced oxidative stress, decreases DNA oxidative damage and prevents the depletion of intracellular GSH in MDCK cells [24].

Keeping in view the importance of *Terminalia arjuna* and the complications arising due to the surgical treatment of kidney stones available, the study was designed to further investigate its antilithiatic potential in vitro and in vivo with the following objectives:

1. To study the aqueous extract of *Terminalia arjuna* on in vitro system of nucleation and aggregation for calcium oxalate and kinetics of calcium oxalate crystal growth.
2. To study the effect of the aqueous extract of *Terminalia arjuna* on the oxalate induced renal epithelial cell injury (NRK-52E and MDCK)
3. To purify, characterize and identify antilithiatic proteins from *Terminalia arjuna* by Peptide mass fingerprinting (PMF) using MALDI-TOF-MS.
4. To study the effect of antilithiatic proteins from *Terminalia arjuna* on the oxalate induced cell injury (NRK-52E and MDCK).
**Figure 1.1** Schematic representation of the bioactivity guided purification and characterization of the antilithiatic proteins from the bark of *Terminalia arjuna*