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## INTRODUCTION

A healthy life is a challenge today in this increasingly competitive world, where we hardly get a chance to care for diet or assure the quality of food we eat. The changing life style made a remarkable shift in the food habits of people all over the world because of increased interest on junk foods and soft drinks. In any industrialized part of the world it is estimated that nearly 10 per cent of the population is affected by urinary tract stone disease (Dharmalingam *et al.*, 2014). The male-female ratio for the occurrence of this disease is 2:1 in the world population. This has led to an alarming situation for the incidences of life style diseases such as diabetes mellitus, arthritis, heart disease and urolithiasis. Urolithiasis is one among the oldest and widely reported diseases known to mankind (Ngo and Assimos, 2007; Sayana *et al.*, 2014).

Urolithiasis or renal lithiasis is also known as urinary calculi, urinary stones, kidney stones, renal stones or renal calculi (singular *calculus*) (Reddy and Vardhaman, 2013). It refers to the growth of hard mineral calcification formed in the urinary system, primarily in the kidney or ureter and may also migrate into the lower parts of urinary system. It is defined as the consequence of an alteration of normal crystallization conditions of urine in the urinary tract, which involves aggregation of minerals from the metabolic process of kidney. This undesirable mineral aggregates cause extreme pain and in some cases this may lead to high blood pressure, increased the risk for coronary artery disease, diabetes mellitus and morbidity at rare instances (Al-Attar, 2010). Hot, arid or dry climate also plays an important role in renal stone formation. Some of the risk factors associated with nephrolithiasis are diet with high oxalate and calcium content, deficiency of vitamin A and excess vitamin D, cystinuria, gout, hyperparathyroidism, intestinal dysfunction and other factors associated with arid climatic conditions leading to insufficient urinary excretion (Kalaitzidis *et al.*, 2014; Diana, 2013).

Stone formation is more common in males than in females, as females tend to experience stone formation after the menopause, which may explain the equal incidence for stone

formation in older people of both sexes. High body mass index is also associated with increased risk of stone formation (Choubey *et al.*, 2010).

Chemical imbalance in the urine decides the chemical composition of stones. Based on their composition, five common types of kidney stones are categorized into calcium oxalate, calcium phosphate, uric acid, struvite, and cysteine stones. Approximately 85 per cent of stones are composed predominantly of calcium compound as calcium oxalate (Stamatiou *et al.*, 2006) and hence calcium oxalate (CaOx) stones are chosen for the present investigation.

The pathophysiological mechanism for calcium based kidney stone formation is complex and diverse, which includes low urine output, hyper-calciuria, hyper-uricosuria, hyper-oxaluria and abnormalities in urine pH (Asplin, 2002). CaOx stones tend to form when the urine is acidic (low pH) and the extent of supersaturation influences the stone formation (Thomas and Howard, 1959; Boyce, 1968). Some of the oxalates in urine is produced by the body under certain physiological conditions. Calcium and oxalate rich diet also plays a part but are not the only factors that cause the formation of calcium oxalate stones. Calcium from bone in certain disease condition may also play a role in kidney stone formation (Curhan *et al.*, 1997; Vamsi and Pujari, 2014).

On the other hand calcium phosphate stones are less common. Calcium phosphate stones tend to form when the urine is alkaline (high pH). Uric acid stones (5-10 per cent occurrence in the population) are more likely to form when the urine is persistently acidic, which may result from a diet rich in animal proteins and purines-substances that are present naturally in all food and particularly in organ meats, fish, and shellfish. ([http://www.uaf.edu/chc/community-resources/educational-resources/NKUDIC\\_KidneyStoneDiet\\_FS.pdf](http://www.uaf.edu/chc/community-resources/educational-resources/NKUDIC_KidneyStoneDiet_FS.pdf)). Struvite stones (5-15 per cent occurrence in population) result from infections in the kidney. Diet has not been shown to affect struvite stone formation. Cystine stones result from a rare genetic disorder that causes cystine, an amino acid leaking through the kidneys, into the urine to form crystals (Moe, 2006).

Kidney stone formation is a complex process from a series of several physico-chemical events including supersaturation, nucleation, growth, aggregation and retention of urinary stone constituents within the renal tubules. As mentioned above, the supersaturation of

urine with stone-forming calcium salts is the basis of calcium stone formation. The accepted management of stone disease ranges from observation to surgical removal of the stone. Various factors namely, the size of the calculi, severity of symptoms, degree of obstruction, kidney function, location of the stone and the presence or absence of associated infection influence the choice of treatment. Stones smaller than 5mm have a high probability of spontaneous passage through urine. However, stones larger than 5mm or stones that fail to pass through are treated by interventional procedures. Open surgical treatment of ureteric stones have gradually disappeared in the last 30 years and was replaced by minimal invasive techniques such as Extracorporeal Shock Wave Lithotripsy (ESWL) or ureteroscopy (Knoll, 2007; Nabi *et al.*, 2007; Laszlo *et al.*, 2014).

ESWL is a non-invasive procedure which uses shock waves to fragment calculi. The success rate of this treatment depends on stone composition, size, properties and location of the stone as well as the intensity of the shock frequency, which may cause deleterious effects on surrounding tissues. Acute renal hemorrhage is the common injury reported during the treatment and at rare instances the complications are fatal (Silberstein *et al.*, 2008; Kumar *et al.*, 2012; Philips *et al.*, 2014).

Effective kidney stone prevention is dependent on the identification of risk factors for stone formation. A high fluid intake reduces urinary saturation of stone-forming calcium salts, leading to dilution of promoters of CaOx crystallization. Vitamin C has been implicated in stone formation because of *in vivo* conversion of ascorbic acid to oxalate and so supplementation should be limited to 500mg/day or less according to Park and Pearle (2007) and Farmanesh *et al.* (2014).

The most effective curative agents for hypocalciuria are thiazide diuretics which enhance calcium reabsorption in the distal renal tubules. Long-term use in 50 per cent of the population however has side effects including fatigue, dizziness, impotence, musculoskeletal symptoms, or gastrointestinal complaints and potassium depletion which causes intercellular acidosis and can lead to hypokalemia and hypocitraturia. Potassium citrate is reported to be effective in the treatment of urinary calcium stones, but a major drawback is its adverse side effects mainly in the gastrointestinal tract and include eructation, swelling and diarrhea (Mattle and Hess, 2005; Moe, 2006; Youssef *et al.*, 2014).

To conclude, none of the listed treatment modalities is without side effects and not provide satisfactory treatment options for urolithiasis. Thus, the focus should be on the development of novel strategies for the prevention and treatment of kidney stone disease. Herbal medicines have optimistic alternative to bridge the gap in this regard. Herbal medicines are increasingly being considered suitable for long-term treatments for renal dysfunction. The search for new antilithiatic drugs based on ethnobotanical approaches therefore assumes greater importance as herbal drugs are not only cost effective but also confer minimal side effects (Rodgers *et al.*, 2014).

Ethnobotany, a scientific discipline which focuses on people-plant relationship in a multidisciplinary manner. Schultes (1992) stated that the relationship between plant and human cultures is not limited to food, clothing and shelter alone but also includes religious ceremonies, ornamentation and health care. Earlier researchers have made outstanding contributions in the ethnobotanical studies to spotlight medicinal virtues of many indigenous plants discovered by traditional healers. Drugs like Quinine, Reserpine, Theophylline, Codeine, Aspirin and Vinblastin are few potential chemical constituents developed in the past based on the ethnobotanical investigations (Diana, 2013).

Immunomodulation, adaptogenic and antimutagenic activities are clinically proven effects of herbal drugs, have created interest among the people. Herbal 'Renaissance' is happening all over the globe and herbs are staging a comeback since the recent past. Synthetic products of the modern age surpassed the importance of herbs for a while; however, the dependence on synthetics is over and people are returning to the naturals with the hope of safety and security. The herbal products today symbolize safety and has been valued for their medicinal, flavouring and aromatic qualities for centuries (Joy *et al.*, 2001). Possible therapeutics for the dissolution of urinary calculi and the subsequent measures for the prevention of its recurrence is the focus of this study.

Pashanabheda/asmabheda group of plants are often prescribed as diuretic and antiurolithiatic drugs traditionally. Pashana/Asma means 'stone', and bheda means the one that breaks. Presently, there are certain medicinal plants namely *Aerva lanata*, *Tribulus terrestris*, *Bergenia ligulata*, *Ammania baccifera*, *Rotula aquatic*, *Coleus aromaticus*, *Nothosarava brachiata*, *Phyllanthus niruri*, *Phyla nodiflora*, *Costus igneus*,

*Triticum aestivum*, *Cumin sativus* and *Homonoia riparia* are being used as drugs across India (Mukhopadhyaya, 1929; Pandey, 2001; Venugopal, 2009; Shelke *et al.*, 2014; Shrinidhi *et al.*, 2014).

Renal cells when exposed to oxalate and CaOx crystals produces reactive oxygen species (ROS), development of oxidative stress followed by injury and inflammation which in turn lead to stone formation. Treatment with antioxidants and free radical scavengers reduce CaOx induced renal injuries and deposition of stones in the kidneys. Various herbs with antioxidative and free radical scavenging property have been reported to inhibit CaOx crystallization (Selvam *et al.*, 2001; Singh and Barjatiya, 2002; Pawar *et al.*, 2009; Kalpana *et al.*, 2013). The need is to develop an effective, safe and standardized herbal formulation for the management of urolithiasis. The present study was undertaken to evaluate the antilithiatic effect of the banana corm (rhizome) of different cultivars, varying in their biochemical composition.

Banana, is a herbaceous plant belonging to the family Musaceae and has its origin in India which is known for its vast varietal wealth. Bananas are cultivated in more than 135 countries throughout the tropics and subtropics. Banana occupies only 11 per cent of the total cultivated area under fruits in India, but it contributes 33 per cent of total fruit production (Mitra, 2014). With a total production of 26.7 million tonnes from 796 thousand hectares, banana contributes 32.6 per cent of the total fruit production in India (Indian Horticulture Database, 2013).

In India banana is referred as ‘Kalpatharu’ a plant of all virtues, known for its antiquity and is interwoven with the national heritage with its multifaceted uses. Each and every part of the plant is used for specific purposes. Traditionally banana corm and pseudostem juice is used to treat people with kidney problems. Stem juice is used as an antidote for snake bite. In the preparation of Siddha medicines, the water extracted from the corm is used. The roots can arrest hemoptysis and possess strongly astringent and anthelmintic properties (Krishna and Amirthalingam, 2014). Banana pulp and peel can be used as natural sources of antioxidants and pro-vitamin A due to their contents in carotenoids, phenolic and amine compounds. For the development of a phytomedicine or even an allopathic medicine e.g., banana fruit pulp and peel could be of interest as raw

material (Pereira and Maraschin, 2015). In order to validate these traditional usage scientifically, the present study involves the use of banana corms of different cultivars as a remedy against stone formation.

The cultivars used in the present study were chosen representing different ploidy levels and genomic composition, including the wild species (Poovan – AAB, Red banana – AAA, Neypoovan – AB, Karpooravalli – ABB, Sannachenkadali – AA, Bhimkol (Wild) – BB). The belief and usage by locals and tribes were also taken into consideration while selecting these varieties, which are also commonly and widely available. Almost all the states in India grows bananas and the varietal preferences vary widely and it is grown round the year. A mature banana plant produces within a year an average of four daughter suckers (maximum of 10 suckers). In one hectare about 2500 plants are planted. The weight of corms including that of the mother plant is almost 10 Kg per clump. One can easily conceive the volume of corms and stems as raw material available for commercial preparation of any products. It is about 25 tonnes per hectare. The raw material can also be procured cheaply (Singh and Chadha, 2000). Thus the present study aims at utilizing these raw materials (banana corm) which are available throughout the year as a source for the preparation of drugs against lithiasis.

### **Hypothesis of the study**

Present study was designed to test the following hypotheses:

**Null hypothesis ( $H_0$ ):** The corm of different banana cultivars do not possess significant antilithiatic activity.

**Alternate hypothesis ( $H_A$ ):** The corm of different banana cultivars contain components that exhibit strong antilithiatic activity.

Hence, the present study was formulated to address the following objectives to test the above mentioned null and alternate hypotheses.

- To screen the selected banana cultivars and a wild species for their antilithiatic potential under *in vitro* conditions
- To evaluate the antilithiatic potential of the banana corm extract with maximum efficacy against lithiasis, under *in vivo* conditions

- To evaluate the *in vitro* antilithiatic potential of the banana corm extract with maximum efficiency using normal rat kidney cells (NRK 52E cells)
- To assess the antioxidant status and to characterize the phytochemical constituents present in the corm extract of selected banana cultivar

The vast literature pertinent to the present study was collected and is briefly reviewed in the next chapter.