INRODUCTION

Disease is a state of discomfort in which the normal functioning of the body is affected. Our biology deals with hundreds of thousands of independent factors which involves complex interaction of all the proteins and cells working with environmental factors. Lifestyle diseases are caused by an inappropriate relationship of people with their environment and are becoming more common as countries are becoming industrialized. With rapid economic development and increasing westernization of lifestyle in the past few decades prevalence of these diseases have reached alarming proportions among Indians in the recent years (Mawale and Pajai, 2014).

Lifestyle diseases are different from other diseases because they are potentially preventable, and can be lowered with changes in lifestyle, diet and environment. Smoking, overuse of alcohol, poor diet, lack of physical activity and inadequate relief of chronic stress are key contributors in the development and progression of preventable chronic diseases, including type 2 diabetes mellitus, obesity, cardiovascular disease, hypertension and several types of cancer (Gupta et al., 2013). Lithiasis is one of the diseases caused by lifestyle changes. Calcium oxalate accumulation is due to intake of cereals rich in calcium and phosphorus besides lack of animal protein and high intake of oxalate rich foods (Rajeshwari and Sudharani, 2013).

Urolithiasis is a stony concretion created by a cluster of crystalline or amorphous particles precipitated in the urine (Hounnasso et al., 2015). Urolithiasis, term coined from the Greek word “ouron” (urine) and “lithos” (stone), is an universal disease. It is the third most frequent problem affecting the urinary tract (Khan and Pradhan, 2012). Urolithiasis affects 10-12% of the world population especially in the industrialized countries. It has a recurrence rate of 50% (Vamsi et al., 2014). The overall probability of lithiasis differs in various
parts of the world depending on the dietary habits and socio-economic conditions. Global warming could be a factor behind the rising number of kidney stone cases reported (Aggarwal et al., 2014).

Urolithiasis, which is referred to as the process of formation of calculi (singular calculus) in the urinary system includes nephrolithiasis (Renal Calculi or Kidney Stones), ureterolithiasis (Ureter Calculi) and cystolithiasis (Bladder Calculi). In urolithiasis, the Glomerular Filtration Rate (GFR) decreases due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood. Urolithiasis is a complex process that results from several physicochemical events including crystal nucleation, aggregation and growth of insoluble particles in the kidney (Khare et al., 2014).

Epidemiological factors, including age, sex, heredity, occupation, body size, social class, affluence, geographic location, climate, diet and fluid intake, have been identified as factors playing key roles in kidney stone disease. Epidemiological studies revealed that urolithiasis is more common in men (12%) than in women (6%) and is more prevalent between the ages of 20-40 in both sex (Sreelakshmi et al., 2014). The symptoms of lithiasis include variable degree of pain in abdomen and the groin, bleeding from urethra, pus in the urine, and further may lead to secondary infection (Tushar et al., 2014). The crystals formed create problems by blocking the flow of urine and cause severe pain termed as renal colic when they move along the ureter (Mikawlrawng et al., 2014).

Some common causes of urolithiasis are inadequate urinary excretion, foreign bodies in the urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities, like Vitamin A deficiencies and Vitamin D excess. Oxalate, the major constituent of the stone formation is very toxic to the renal epithelial cells. Studies show that the exposure of the cells to oxalate leads to disruption of the normal activities of the renal epithelial cells such as altered membrane surface properties and cellular lipids, changes in gene expression, disruption of mitochondrial function, formation of reactive oxygen species and
In vitro and in vivo investigation of antilithiatic and antioxidant activity of aqueous extract of *Aerva lanata* decreased cell viability (Aggarwal *et al.*, 2010a). Patients suffering from diseases like hyperparathyroidism, renal tubular acidosis, cystinuria, hypercalciuria, hyperoxaluria and Crohn’s disease are more prone to stone formation. Reduction in urine volume, elevated amount of solute in urine, fluctuation in urinary pH and infection may also lead to stone formation (Vyawahare *et al.*, 2014).

Oxidative stress plays an important role in stone formation caused by the excess production of Reactive Oxygen Species (ROS). ROS are various forms of activated oxygen, which include free radicals such as superoxide ions ($O_2^-$) and hydroxyl radicals (OH) as well as non-free radical species such as hydrogen peroxide ($H_2O_2$). Most ROS are fleeting and cannot travel long distances hence oxidative stress usually materialize as the excessive by-products of ROS interaction with lipids, amino acids, proteins, carbohydrates and nucleic acids. This stress causes damage to the epithelium of the kidney or bladder producing a constructive environment for crystal attachment. It is observed that in the absence of tissue injury there was no crystal retention. The cell or tissue injury exposes molecules on the cell surface that are not usually open for the crystals resulting in adhesion of the crystals (Singla *et al.*, 2013).

Calcium-containing stones, especially calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate are the most commonly occurring ones to an extent of 75-90% followed by magnesium ammonium phosphate (Struvite) (10- 15%), uric acid (3-10%) and cystine (0.5- 1%). Crystal nucleation, growth and agglomeration in urine are believed to be controlled by a balance between the ambient physico-chemical supersaturation and inhibitors capable of acting on one or more of these crystallization steps. The imbalance in the promoters and inhibitors will increase the risk of stone formation (Gupta *et al.*, 2011).

If stones are small in size, they usually pass in the urine unnoticed. Diagnosis of the urinary stone is usually done by X-ray or ultrasound in patients who complain of blood in urine and sudden pain. These diagnostic image gives the doctor important information on the size and location of the stones. There are some parameters that indicate the biochemical changes that have occurred in
the urine and blood of the stone formers. Recently, Computerized Tomography (CT) and Intravenous Pyelogram (IVP) or intravenous urogram has been used for diagnosis (Malan et al., 2011).

The main goals for urinary stone treatment are to preserve renal function, reduce or avoid complications related to calculi, and to render the patient free of calculi as soon as possible. Procedures with low morbidity and rapid recovery are also essential in current practice (Abreu et al., 2015). Potassium citrate, allopurinol, thiazides are some of the drugs that have been used for the medical management of stone formation and also to increase diuresis (Polo et al., 2013). These drugs used for management of the stones are usually not equally effective in all patients and can also lead to adverse side-effects (Jafar et al., 2012).

Off late the medical management of kidney stones is dependent on surgical treatments like Extracorporeal Shock Wave Lithotripsy (ESWL), Percutaneous Lithotripsy (PNL), and Transureteral Lithotripsy. These may lead to acute renal injury, reduction in renal function, hypertension and hemorrhage and do not cause noticeable effect on the recurrence of kidney stone (Mandavi et al., 2012; Xu et al., 2013). The need for a medical treatment that confers both curative and preventive effect against stone formation without side effects led the scientists to turn their attention towards traditional medicine.

Plants are a rich source of many natural products. In India, plants have been used for a long time in traditional medicine like Ayurveda, Unani and Siddha. The vast majority of people in the world take care of themselves using the healing powers of plants that have been used for hundreds of years (Immanuel and Elizabeth, 2009). Medicinal plants, therefore have important contribution in the primary healthcare systems of local communities as the main source of medicines for the majority of the rural population (Amri, 2014). The medicinal value of herbal drug is due to the presence of certain chemical substances in these plants. These natural compounds physiologically affect the human body, interact with the disease causing compound or pathogens, their growth and function at different stages of development and make the body
disease free (Mittal et al., 2014). In the last decade, there has been considerable interest in resurrecting medicinal plants in western medicine, and integrating their use into modern medical systems. The reasons include low cost, nutritional value, drug resistance, less side effects, easy availability and economical value (Sarma et al., 2014).

The study of medicinally important weeds has not been realized as fully as other traditional plants. Weeds are a global, ubiquitous influence of agriculture (Mukherjee, 2009). A weed, in a general sense, is a plant usually wild or untamed that is commonly considered to be a nuisance in a garden, lawn, or other agricultural development. More specifically, the term is often used to describe plants that grow and reproduce aggressively. They do not depend on human intervention for reproduction and survival. Weeds are now widely regarded as pests of crop because they lower the yield and increase the cost of production of desirable crops in various ways. Weeds are usually considered plant pest for two reasons, it is one of the two major threats to biodiversity, second to habitat loss (Grace et al., 2004, Larbie et al., 2014).

Weed species are adapted to fires, lack natural enemies, grow faster than indigenous species and produce plenty of seeds. Many weeds contain compounds which are biologically active and have potential use in medicine. Biochemical evidences supports the theory that plants growing in disturbed areas have more chemicals in them for defense. In spite of evidences, the significance of weeds as an important source of medicine and cure for indigenous people are overlooked (Panda et al., 2015). Earlier studies on the various parts of the weeds showed the presence of secondary metabolites like alkaloids, saponins, flavonoids, glycosides, tannins, terpenoids and phenolic compounds which are responsible for various biological activities (Rao et al., 2011). Alkaloids, saponis and tannins are good antibiotic agents (Thamaraiselvi and Jayanthi, 2012). Flavonoids are powerful water soluble free radical scavengers and wonderful antioxidants which prevent oxidative cell damage (Abbas et al., 2013).
The term “Phytochemicals” refers to a wide variety of compounds made by plants, mainly those that affect human health. These are present in virtually all the fruits, vegetables, legumes and grains we eat. These are a large reservoir of phytochemical compounds, including secondary metabolites (Bohlin et al., 2010). The secondary metabolites are stored in low concentration in the plant tissue and play an important role in the survival of the plant in the environment and are rich source of bioactive compounds that are biodegradable into non-toxic products. Phenols, terpenes, alkaloids, steroids, flavonoid and saponins are considered the main groups of secondary metabolites and are classified according to their biosynthetic pathway (Bernabe-Antonio et al., 2014).

Phytochemicals are non-essential and non-nutritive plant chemicals. These phytochemicals either alone or in combination have tremendous therapeutic potential in curing diseases or disorders such as cancer, coronary heart diseases, diabetes, high blood pressure, microbial protection, ulcers, osteoporosis, kidney stone diseases and related disorders. Phytochemicals have either antioxidant or hormone like actions (Prakash et al., 2012).

The term ‘antioxidant’ refers to the activity of numerous vitamins, minerals and phytochemicals which provide protection against the damage caused by ROS. Antioxidants interfere with the oxidative processes by scavenging free radicals, chelating free catalytic metals and by acting as electron donors. The natural antioxidant mechanisms may be insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important (Padmanabhan and Jangle, 2012).

The medicinal value of plants lies in the bioactive phytochemicals present in the plant. Through phytochemical screening, one could detect the various important compounds which could be used as base of modern drug for curing diseases (Sheikh et al., 2013). Qualitative phytochemical analysis of the plants confirms the presence of various secondary metabolites. This preliminary screening draws attention to the need for further studies of the active principles identified for the treatment of many diseases in order to understand their mode of action in controlling diseases (Singh et al., 2012).
The present study focuses on weeds such as *Tribulus terrestris, Aerva lanata, Scoparia dulcis and Tridax procumbens* which have been used in folk medicines to cure lithiasis. The study focuses on establishing scientific validation for the use of these selected weeds with maximum efficacy to cure lithiasis.

**Hypothesis of the study**

Present study was designed to test the following hypotheses:

**Null hypothesis (H₀):** The various extracts of the selected medicinal weeds do not possess significant antilithiatic activity.

**Alternate hypothesis (Hₐ):** The extracts of the selected medicinal weeds contain active compounds that confer 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 and compare the selected weeds for their antilithiatic potential under *in vitro* conditions
- To evaluate the antilithiatic potential of the weed extract with maximum efficacy using male albino Wistar rats and NRK 52E cell lines
- To assess the antioxidant status and phytochemical determination of the selected weed extract

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