Analysis of kidney stones by PXRD and evaluation of the antiurolithic potential of *Coix lacryma jobi*.

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Abstract- From centuries human has been afflicted by urinary calculi (Urolithiasis). These calculi are formed from crystal aggregation and retention in the urinary tract. The incidence and prevalence of urolithiasis may be related to various factors including age, sex, ethnicity, diet, fluid intake, genetic predisposition, climatic conditions, and urinary tract infections. Study of chemical composition and ultrastructure of various types of calculi helps in understanding mechanism of urolith nucleation, growth and aggregation; subsequently, it helps in prevention and management of urolithiasis. In the present investigation, PXRD technique was used to determine mineral composition of renal calculi. Results of the present investigation revealed that renal calculus of calcium oxalate is more abundant than calcium salts of phosphate. Among the calcium salts of oxalate, whewellite form is more abundant than weddellite form. From centuries back, medicinal plants were used for the treatment of urolithiasis. Here in the present investigation, antiurolithic property of a commonly used medicinal plant, *Coix lacryma jobi* was assessed by subjecting different extracts of this plant for its *in vitro* antibacterial activity and decrystallizing property. Among the different extracts, extracts of chloroform and petroleum show maximum antibacterial property. Moreover, the aqueous extract can decrystallize renal calculi. Therefore, *Coix lacryma jobi* may be used as a plant for the treatment of urolithiasis. The present investigation also highlights that calcium oxalate are found to be more prevalent than other types of renal calcluli. However, mechanism of decrystallizing property and antibacterial activity of this plant needs further investigation.

Index Terms- Urolithiasis, Antibacterial, Renal calculi, Decrystallization.

I. Introduction

Urolithiasis, the formation of urinary calculi anywhere in the urinary system, is one of the common disease of urinary tract and can cause renal failure in human (1). The incidence and prevalence of the urolithiasis may be related to various factors including age, sex, ethnicity, diet, fluid intake, genetic predisposition, climatic conditions and urinary tract infections (2). It has been recognised as one of the most painful medical disorders having the tendency to recur, and the recurrence rate is 75% during 20 years (3). Approximately 80% of stones are composed of calcium oxalate and calcium phosphate, 10% of struvite, 9% of uric acid and the remaining 1% composed of cystine or ammonium acid irate (4). Apart from various factors which induce the formation of kidney stones, kidney stone formation by bacterial infection in the urinary tract is one of the serious problem due to its high prevalence of recurrence. Most of the urea splitting organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Escherichia coli* were reported to be capable of causing urinary tract infection (5),(6). Bacterial infection may induce stone formation by cystal adherence. When the urea-splitting organisms infect the urinary tract, bacteria disintegrate the urea excreted in the urine in the presence of urease enzyme, which subsequently trigger the formation of ammonia rendering the urine alkaline. In alkaline state, urine tends to contain precipitated crystals of calcium and magnesium phosphate and calcium carbonate in large amount thereby leading to a strong tendency to form calcium phosphate and calcium carbonate calculi (7). For the prevention and management of urolithiasis, studies of chemical composition and ultrastructure of various types of kidney stones is very important. Various techniques can be used to determine the mineral composition of the kidney stones. The PXRD technique can be used as one of the most efficient method to determine mineral composition of the renal calculi (8). From centuries plants have been used for the treatment of various ailments. About 80% of the world population depends on medicinal plants for their primary health care (9). From the last three decades the use of medicinal plants is increased as people thought that medicinal plants has less side effects and are more safer than the modern allopathic medicines (10),(11). A variety of plants including those used by traditional medical practitioners grow luxuriantly in Manipur, a region in the North-Eastern part of India, which happens to be within the Indo-Burmese mega-biodiversity hot-spot (12). Many plants are conventionally used to prevent formation of stone as well as to dissolve and remove them from the human body. Among them *Coix lacryma jobi*, a tall grain-bearing tropical plant belonging to the family Poaceae have been used from the time immemorable by local traditional healers in the treatment of kidney stone and Urinary tract infection (13). But no scientific investigation had been carried out. The present study aims to investigate the antiurolithic property, antimicrobial property and kidney stone solubility test of the different extracts of *Coix lacryma jobi*. Moreover, present investigations also perform the kidney stone analysis using PXRD method.

II. Materials and Method

2.1 Collection and preparation of plant extracts:

The leaves of *Coix lacryma-jobi* Var. were randomly collected from different locations of Imphal East and Imphal West Districts of Manipur, India. The leaves were washed with tap water, then rinsed with distilled water and cut into small

pieces and then subjected to shade dry at 28°C. Then the leaf-pieces were crushed into fine powder with mortar and pestle. 150gm of the powder was subjected to extraction with different solvents (aqueous, methanol, chloroform, hexane, petroleum ether) having 2.5 L each for each solvent at 60-80°C by continous hot percolation using Soxhlet extractor. The extraction process was continued for 24 hour. The crude extract was separated using Rotar vapour evaporator. The semi solid fractions were redissolved in Dimethyl sulfoxide (DMSO) and were kept at 4°C until further use.

2.2 Analysis of Kidney stones by PXRD:

Renal calculi were collected from seven urolithic patients who have done litrostropic surgery at Department of Urology, Regional Institute of Medical Sciences, Manipur. The determination of the qualitative and quantitative analysis of chemical compounds present in the kidney stones was performed by PXRD at Indian Institute of Chemical Technology (IICT), Hyderabad.

2.3 *In vitro* antimicrobial activity assay:

Antibacterial activity was determined by standard filter-disc diffusion technique. The *in vitro* antimicrobial activity was performed against overnight grown cultures of three selected bacteria, namely *Escherichia coli*, *Klebsella pneumonia* and *Pseudomonas aeruginosa.* Gentamicin was used as positive control in this experiment. The bacterial cultures were maintained on slants consisting of nutrient agar medium. 48 hour cultures of *E. coli*, *K. pneumoniae* and *P. aeruginosa* were used in the *in vitro* antimicrobial activity assay. 0.05 mg of each extract was dissolved separately in 1ml sterile of dimethyl sulfoxide (DMSO). Nutrient agar medium was prepared and sterilized by pressure cooker. In an aseptic room, inside the laminar flow they poured unto sterile petridishes to a uniform depth of 3mm and then allowed to solidify at room temperature for overnight for checking of contamination. Next day after solidification the test organisms were inoculated with the help of L-shape spreader a bacterial culture of suspensions. Thus provides the uniform surface growth of bacterium and is use for antibacterial sensitivity studies. The wells (6mm in diameter) were dug in the media with the help of a sterile metallic borer. The recommended test sample (0.05gm/1ml in DMSO) was introduced in the respective wells. The plates were incubated immediately at 37°C for 48 h. Microbial growth inhibition was determined by measuring the diameter of the zone of inhibition which was assessed at 48 h incubation.

2.4 Solubility test of Kidney stones:

Solubility test of the renal calculi were performed by keeping the kidney stones to the aqueous extract of *Coix lacryma-jobi*. 0.05 gm of aqueous extract of plant was dissolved in 1ml of distilled water to make a solution. Three stones were taken and measured their weight (W1 = 0.038g, W2 = 0.050g, W3 = 0.050g) before putting to the aqueous extract of *Coix lacryma-jobi* Var. The stones were kept in separate sample tube after proper labelling. Then 1ml of the solution were added and kept at room temperature for one week. After one week, the supernatant were removed from the 3 test tube and then transferred to another three different test tube for further investigation. The stone have been taken out and wipe with the filter paper until it was dry and then measured their weight.

III. Results

3.1 Analysis of Kidney stones by PXRD:

X-ray diffraction analysis was used for identifying the crystalline components of renal calculi. Studies of chemical composition and ultrastructure of various types of renal calculi using X-ray diffraction analysis can help to understand the mechanism of urolith nucleation, growth and aggregation and subsequently help in the prevention and management of urolithiasis. In the present investigation, PXRD technique was used to determine mineral composition of the renal calculi. Results from the PXRD studies revealed that the calcium oxalate, represented by Whewellite and Weddellite mineral shown in figure 1, is the most dominant type of the renal calculi under investigation, in addition to other minerals such as calcium phosphate and Hydroxy apatite. The chemical formula and mineralogical name of the renal calculi are shown in the Table 1.

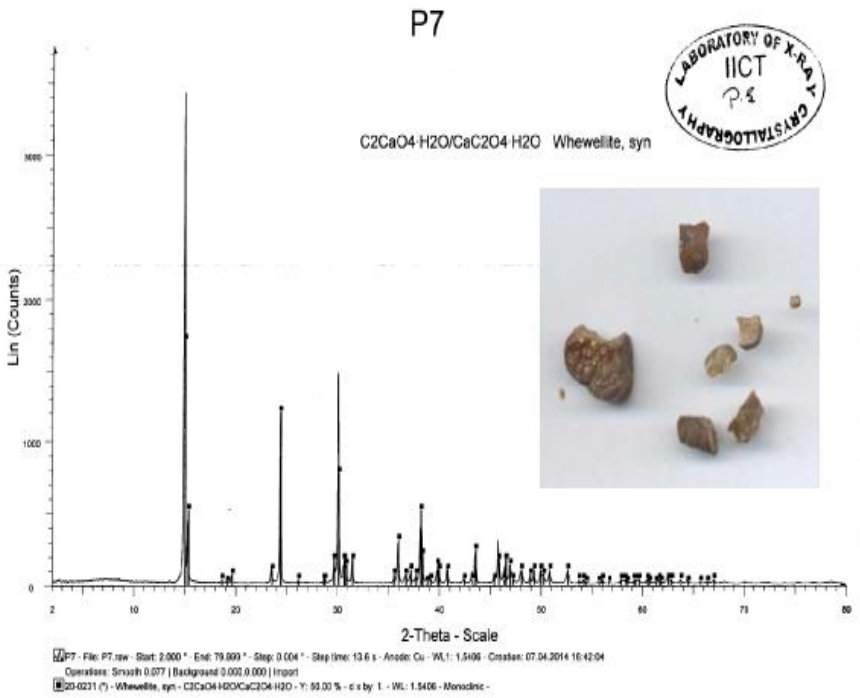

Figure 1: Photograph showing the PXRD data of Whewellite

Table1: Chemical formula and mineralogical name of renal calculi

Formula	Mineralogical Name
$Ca_2CaO_4.H_20/CaC_2O_4.H_20$	Whewellite
$CaC_2O_4(H_2O)_2$	Weddellite
$CaHPO_4$	Calcium hydroxide phosphate
$Ca_{10}(OH)_2(PO_4)/10CaO.3P_2O_3.H_2O$	Hydroxy apatite
$Ca_2P_2O_7$	Calcium phosphate

3.2 *In vitro* antimicrobial activity assay:

The efficacy of the antibacterial activity of the five different extracts of *Coix lacryma-jobi* (aqueous, methanol, chloroform, hexane and petroleum ether) were tested against *Escherichia coli*, *Klebsella pneumonia* and *Pseudomonas aeruginosa*. The antibacterial activity tested against *Klebsella pneumonia* shows maximum efficiency in the chloroform extract followed by petroleum ether extract, aqueous extract, hexane extract and methanol extract. The antibacterial activity tested against *Klebsella pneumonia* also shows maximum efficiency in the chloroform extract followed by petroleum ether extract, aqueous extract, methanol extract and hexane extract. However the antibacterial activity tested against *E.coli* do not show any activity i.e. couldn't inhibit the growth of microbe. The antibacterial efficacy of the different extracts of *Coix lacryma-jobi* were compared with Gentamicin. The results obtained from the present investigation reveals that the non polar extracts (chloroform and petroleum ether) of *Coix lacryma-jobi* have highest antibacterial efficacy than the polar extracts (aqueous and methanol) of *Coix lacryma-jobi.* The effect of plant extracts of *Coix lacryma-jobi* on *Klebsella pneumonia* and *Pseudomonas aeruginosa* were shown in Figure 2.

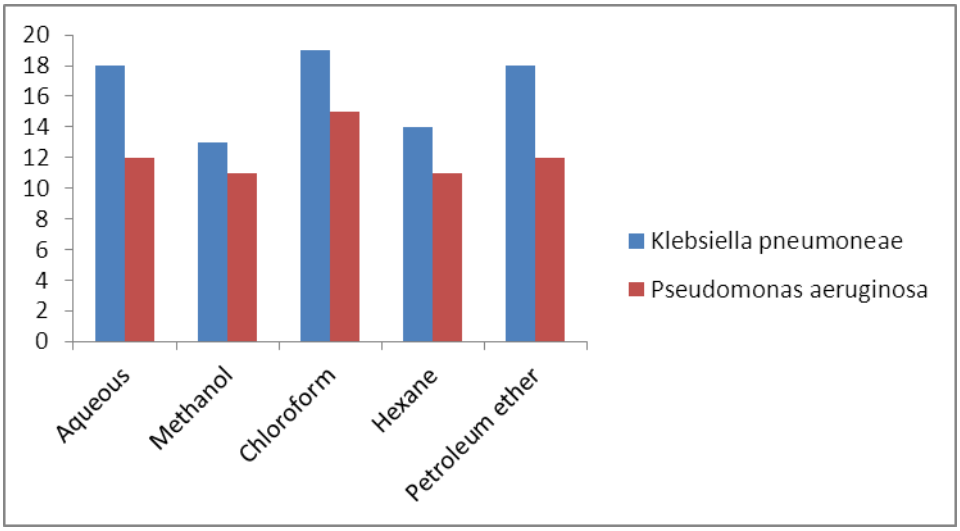

Figure 2: Effect of plant extracts of *Coix lacryma-jobi* on *Klebsella pneumonia* and *Pseudomonas aeruginosa*

3.3 Solubility test of renal calculi:

The effect of the aqueous extract of *Coix lacryma-jobi* on renal calculi decrystallization activity was investigated. Results from the present study observed a siginificant positive effect on renal calculi decrystallization in almost all the tested samples (Table 2).

Table2: Comparing the weight of renal calculi

Weight of renal calculus before putting the aqueous extract of *Coix lacryma-jobi*	Weight of renal calculus after putting the aqueous extract of *Coix lacryma-jobi*
0.038g	0.036g
0.050g	0.048g
0,050g	0.036g

IV. Discussions

Renal calculi have been recognized as one of the most painful medical disorders. The incidence of renal calculi formation also increased with the years to come. Identification of the chemical constituents of renal calculi is important in the diagnosis and management of urolithiasis. The compositional variability of uroliths has different etiologies and requires various mode of treatment and prophylaxis. The results of the present study shows identification of chemical constituents of renal calculi by PXRD. Results from the PXRD studies revealed that the calcium oxalate, represented by whewellite and weddellite mineral shown in figure 1, is the most dominant type of the renal calculi under investigation,in addition to other minerals such as calcium phosphate and hydroxy apatite. The control of urolithiasis depends on the active biomolecules present in the medicinal plants. This eventually would lead to a break-through in the prevention of urolithiasis and may lead to the discovery of novel bioactive molecules which can be helpful in the management and control of urolithiasis.(13) Thus, this investigation would further open up new avenues to the use of these medicinal plants in drug development for the treatment of urolithiasis.

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