# Antiurolithiatic Property of *Pratia Nummularia* Kurz, *Cyperus Rotundus* Linn and *Citrus Latipes* and Comparison of Their Antiurolithiatic Properties with Cystone, a Herbal Drug

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***ABSTRACT***

*Medicinal plants are used to combat diseases from the dawn of civilization. These plants have rich resources of therapeutic agents for the prevention of various ailments like urolithiasis, diabetes, hypertension etc. Urolithiasis is the condition where urinary calculi are formed in the tract. It is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70-80 % in males and 47-60% in females. It causes health problems such as severe pain, urinary tract obstruction and infection that adversely affect well being individuals. Treatment option includes shock wave lithotripsy, ureterocopy, open or laproscopy stone removal etc. which are costly and painful. Many synthetic drugs are used for the antilithiasis but overuse of synthetic drugs which results in the higher incidence of adverse drugs reactions have motivated humans to return to nature for safe remedies. Medicinal plants have less side effect and more economically viable . In the present paper, an attempt has been made for the antiurolithiatic property of some plants in the vitro condition i.e. one in the aqueous medium and other in the urinary medium. And also observe the comparison of their antiurolithiatic properties with Cystone, a herbal drug.*

**Keywords**: *Medicinal plants, Antiurolithiatic, Lithotripsy, Ureterocopy, Diabetes, Herbal drug*

## I. INTRODUCTION

Medicinal plants are used to combat many diseases from the dawn of civilization. These plants are the rich source of therapeutic agents for the treatment and prevention of many ailments. These plants and their traditional use are the most economic and low side effective method of choice for the treatment of many diseases and ailments for the worldwide .India is one of the world's 12 biodiversity centers with the presence of over 45000 different plant species [1]. Many of these plants are rare and endemic and found only in the forest regions. Some plants are very effective in the antiurolithiatic property.

Stone formation in the kidney and gall bladder is very common now-a-days. It is estimated that 12% world population experience renal stone diseases with a recurrence rate of 70-80% in male and 47-60% in female [2,3]. Urinary calculi are the third most common affliction of the urinary tract which are exceeded by the urinary tract infections and prostate diseases [4].

Kidney stone diseases is a most ancient and common affliction in human beings with about 1-15% of the world population affected. No age is spread and no country or ethnic group is protected from this common clinical problem. Although a few individuals die as a direct consequence of stone diseases, it does lead to substantial morbidity from pain, urinary tract infection and obstructive uropathy. Now-a-days people know the effective programme for both treatment and prevention of stone diseases .

Factors responsible for the stone formation include age, sex, occupation, social class, season of

the year and climate, dietary and fluid intake and genetic predisposition [6,7].Genetic predisposition is essential endorsed by the most frequent episodes of stone formation in the family members of stone formers as compared to non-stone formers. Nevertheless some environmental factors (likely to be dietary habits) shared by family members are believed to be relatively more important that genetic predisposition. A hot sunny climate may influence stone formation through inducing dehydration with increased perspiration and increased solute concentration with decreased urine volume, coupled with inadequate liquid intake. This is possibly through the greater exposure to UV radiation which eventually results in an increased vitamin D production conceivably correlated with seasonal variation in Ca and excretion to the urine. Therefore stone formation is a process that occurs due to imbalance between promotion and inhibitors in the kidney[8]. Hence in brief, factors affecting stone are urine output(hence the concentration). The concentration of specific constituent, urine $p^H$, infection or damage within the urinary tract [9].

Physicians usually do not treat kidney stone, rather they just medicate the pain until the stone pass out their own. The easiest way is the open renal surgery. But the vegetarian diet, heavy on herbs and liquids, can be helpful in the prevention and treatment of kidney stone. So the best way to prevent kidney stone is to drain plenty of water and take a vegetarian high in magnesium. Hence the best safety way for the treatment of stone is the use of medicinal plants. In Manipur, the north east state of India, traditional knowledge for the treatment of stones in kidney and its tract are widely used. In such treatment, aqueous extract of medicinal plants are mainly used. The present study is focused on the collection of crude from the aqueous extract of the plants and study of their chemoinhibitory effects on mineralization of calcium phosphate and calcium oxalate in urinary medium in vitro condition.

Different experimental procedures have been proposed to study growth and inhibition of stone formation in vitro using synthetic diluted or natural supersaturated aqueous solution of urine are taken. Crystallization is triggered by adding calcium oxalate and phosphate to the reaction medium. The effects of hydromethanolic extracts of *Pratia nummularia* Kurz [10], *Cyperus rotundus* Linn [10] and *Citrus latipes* [10] were studied on the growth and inhibition of calcium oxalate monohydrate(COM) and calcium phosphate(CP) in vitro. In the vitro study, we show that plant extracts are used in urine owing to its therapeutic potential as a preventive agent by hindering the formation of COM and CP crystals.

## II. EXPERIMENTAL

Manipur is rich in medicinal plants. The healthy plants(leaf, whole parts and fruit) of *Pratia nummularia* Kurz, *Cyperus rotundus* Linn and *Citrus latipes* are collected from the different parts of Manipur. The herbarium of the plants is already reported. The parts of the plants were washed, dried, chopped and powdered. In the meantime Cystone, a herbal drug, manufactured by the Himalayan company, was procured from the pharmacy. The dried powdered leaf and fruit of the three plants were socked in 50% aqueous methanol in a soxhlet extractor under hot condition[11,12]. The extract were distilled under reduced pressure using Rotary Vacuum Evaporator(RII) to produce crude mass which further spread in Petridis and kept in desiccators.

Table I*: Medicinal plants with scientific and local names and plant used parts.*

| SL.No. | Scientific name | Local Name | Part used |
|---|---|---|---|
| 1 | *Pratia nummularia* Kurz | Kihomman | Leaf |
| 2 | *Cyperus rotundus* Linn | Sembang Kaothum | Whole parts |
| 3 | *Citrus latipes* | Heiribob | Fruit |

### *A. Collection of Urine*

Urine was collected from a healthy male(30 years) who does not have any stone cases, in a sterilized container and camphor was added as preservative. It is so required just as solvent to mimic the natural solvent system. The freshly collected urine was always used in the experiment.

Water contents of the three plants were determined and are shown in Table II.

Table II: *Water content*

| Sl.No. | Plants | Parts | Mass of plant extract before drying(g) | Mass of plant extract after drying(g) | Mass of water content(g) |
|---|---|---|---|---|---|
| 1 | *Pratia nummularia* Kurz | Leaf | 3.2059 | 0.7240 | 2.4810 |
| 2 | *Cyperus rotundus* Linn | Whole part | 1.0260 | 0.2670 | 0.7590 |
| 3 | *Citrus latipes* | Fruit | 2.6340 | 0.2580 | 2.3760 |

Inhibitory experiments of the plants including blank reading both in aqueous and urinary media were performed. All the inhibition experiments were performed according to T.V.RK Rao [13]. 0.01M each of $CaCl_2$ and $Na_3PO_4$ were taken for CP crystallization. Similarly 0.01M each of $CaCl_2$ and $Na_2OX$ were taken for COM crystallization. 50ml of plant extract(PE)(0.01% of crude) in water or urine was taken as inhibitor solutions. Simultaneous blank experiments with water or urine in place of inhibitor solution were also carried out for evaluating the inhibitor efficiency of inhibitors compared to water or urine(Table III&IV). All the experiments were conducted at room temperature (25$^{o}$C). At the end the content of the beaker were digested on a hot water bath for 10 minutes, cooled at room temperature and centrifuged in small volume. The total centrifugates were collected. Calcium content of the centrifugate, left after stone had formed, was determined by complexometric titration using standard EDTA solution(0,01M) [14], EBT(1%) indicator and $NH_3$ – $NH_4Cl$ as buffer($p^H$-10).While calculating the Ca content of the centrifugate, a titre value of EDTA versus corresponding total inhibition solution was deduced from the total titre value(equivalent to centrifugate)(Table V to XIII). Inhibition efficiency was calculating by using the following equation.

Inhibition efficiency(i.e.% Inhibition) = $\frac{Ca^{2+} \text{ in centrifugate}}{\text{T0tal } Ca^{2+} \text{inthe experiment}}$

Thus, % increase of inhibition efficiency relative to blank = $\frac{\text{Increase of \% inhibition over blank}}{\text{\% Inhibition by blank}}$

When the total $Ca^{2+}$in the experiment equals the $Ca^{2+}$contents of 50ml $CaCl_2$ solution which was determined separately. The experimental findings are shown in Tables(III to VII)

Table III*: Inhibition experiment for CP formation in aqueous and urinary media*

| | Aqueous medium for CP formation | | | | Urinary medium for CP formation | | | |
|---|---|---|---|---|---|---|---|---|
| Sl.No. | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) |
| 1 | 0 | 10.3 | 10.3 | | 0 | 10.9 | 10.9 | |
| 2 | 0 | 10.2 | 10.2 | 10.2 | 0 | 10.8 | 10.8 | 10.8 |
| 3 | 0 | 10.2 | 10.2 | | 0 | 10.8 | 10.8 | |

Table IV*: Inhibition experiment for COM formation in aqueous and urinary media*

| | Aqueous medium for COM formation | | | | Urinary medium for COM formation | | | |
|---|---|---|---|---|---|---|---|---|
| Sl.No. | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) |
| 1 | 0 | 1.2 | 1.2 | | 0 | 2.6 | 2.6 | |
| 2 | 0 | 1.2 | 1.2 | 1.2 | 0 | 2.5 | 2.5 | 2.5 |
| 3 | 0 | 1.2 | 1.2 | | 0 | 2.5 | 2.5 | |

Table V*: Inhibition experiment for Pratia nummularia Kurz*

| | PE(0.1%) in aqueous medium for CP formation | | | | PE(0.1%) in urinary medium for CP formation | | | |
|---|---|---|---|---|---|---|---|---|
| Sl.No. | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) |
| 1 | 0 | 9.0 | 9.0 | | 0 | 10.5 | 10.5 | |
| 2 | 0 | 8.9 | 8.9 | 8.9 | 0 | 10.4 | 10.4 | 10.4 |
| 3 | 0 | 8.9 | 8.9 | | 0 | 10.4 | 10.4 | |

| | PE(0.1%) in aqueous medium for COM formation | | | | PE(0.1%) in urinary medium for COM formation | | | |
|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 10.0 | 10.0 | | 0 | 5.4 | 5.4 | |
| 2 | 0 | 9.9 | 9.9 | 9.9 | 0 | 5.3 | 5.3 | 5.3 |
| 3 | 0 | 9.9 | 9.9 | | 0 | 5.3 | 5.3 | |

Table VI: *Inhibition experiment for Cyperus rotundus Linn*

| | PE(0.1%) in aqueous medium for CP formation | | | | PE(0.1%) in urinary medium for CP formation | | | |
|---|---|---|---|---|---|---|---|---|
| Sl.No. | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) |
| 1 | 0 | 10.0 | 10.0 | | 0 | 12.6 | 12.6 | |
| 2 | 0 | 9.9 | 9.9 | 9.9 | 0 | 12.5 | 12.5 | 12.5 |
| 3 | 0 | 9.9 | 9.9 | | 0 | 12.5 | 12.5 | |
| | PE(0.1%) in aqueous medium for COM formation | | | | PE(0.1%) in urinary medium for COM formation | | | |
| 1 | 0 | 2.5 | 2.5 | | 0 | 6.3 | 6.3 | |
| 2 | 0 | 2.4 | 2.4 | 2.4 | 0 | 6.2 | 6.2 | 6.2 |
| 3 | 0 | 2.4 | 2.4 | | 0 | 6.2 | 6.2 | |

Table VII*: Inhibition experiment for Citrus latipes*

| | PE(0.1%) in aqueous medium for CP formation | | | | PE(0.1%) in urinary medium for CP formation | | | |
|---|---|---|---|---|---|---|---|---|
| Sl.No. | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) | IR(ml) | FR(ml) | Diff.(ml) | Mean(ml) |
| 1 | 0 | 9.1 | 9.1 | | 0 | 15.6 | 15.6 | |
| 2 | 0 | 9.0 | 9.0 | 9.0 | 0 | 15.5 | 15.5 | 15.5 |
| 3 | 0 | 9.0 | 9.0 | | 0 | 15.5 | 15.5 | |
| | PE(0.1%) in aqueous medium for COM formation | | | | PE(0.1%) in urinary medium for COM formation | | | |
| 1 | 0 | 2.0 | 2.9 | | 0 | 7.1 | 7.1 | |
| 2 | 0 | 1.9 | 1.9 | 1.9 | 0 | 7.0 | 7.0 | 7.0 |
| 3 | 0 | 1.9 | 1.9 | | 0 | 7.0 | 7.0 | |

Table VIII: *Effect of Blank solution on CP formation*

| Sl.No. | Solvent | BR | $Ca^{2+}$ in solution(g) | $Ca^{2+}$ in precipitate(g) | % Inhibition |
|---|---|---|---|---|---|
| 1 | Water | 10.2 | 0.0008x10.2 =0.00816 | 0.07351-0.00816 =0.06545 | 0.00816x100/0.07351 =11.1005 |
| 2 | Urine | 10.8 | 0.0008x12.8 = 0.00864 | 0.07351 –0.00864 = 0.06487 | 0.00864x100/0.07351 =11.7535 |

Table IX: *Effect of PE on CP formation in aqueous medium*

| Sl. No. | Plant name | Inhibitors 0.1% | $Ca^{2+}$ in solution(g) | $Ca^{2+}$ in precipitate(g) | % of Inhibition | Diff. in % of inhibition between sample and blank | Relative % of inhibition |
|---|---|---|---|---|---|---|---|
| 1 | *Pratia nummularia* Kurz | 8.9 | 0.0008x8.9 = 0.00712 | 0.07351-0.00712 =0.0664 | 0.00712x100/0.07351 = 9.6858 | 9.6858-8.924 =0.7622 | 0.7622x11.2058 =8.5411 |
| 2 | *Cyperus rotundus* Linn | 9.9 | 0.0008x9.9 = 0.00792 | 0.07351-0.00792 = 0.0656 | 0.00792x100/0.07351 =10.7740 | 10.7740-8.9240 =1.8501 | 1.8501x11.2058 =20.7319 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3 | *Citrus latipes* | 9.0 | 0.0008x9.0 =0.0072 | 0.07351-0.0072 =0.0663 | 0.0072x100/0.0735 1 =9.7946 | 9.7946-8.9240 =0.8706 | 0.8706x11.205 8 =9.7558 |

Table X *: Effect of PE on CP formation in Urinary medium*

| Sl. No. | *Plant name* | Inhibitors 0.1% | $Ca^{2+}$ in solution(g) | $Ca^{2+}$ in precipitate(g) | % of Inhibition | Diff. in % of inhibition between sample and blank | Relative % of inhibition |
|---|---|---|---|---|---|---|---|
| 1 | *Pratia nummularia* Kurz | 10.4 | 0.0008x10.4 =0.0083 | 0.07351-0.0083 =0.0652 | 0.0083x100/0.07351 = 11.2910 | 11.2910-11.1005 =0.1905 | 0.1905x100/11.1005 =1.7161 |
| 2 | *Cyperus rotundus* Linn | 12.5 | 0.0008x12.5 =0.0100 | 0.07351-0.0100 =0.0635 | 0.0008x100/0.07351 = 13.6036 | 13.6036-11.1005 =2.5031 | 2.5031x100/11.1005 = 22.5494 |
| 3 | *Citrus latipes* | 15.5 | 0.0008x15.5 = 0.0124 | 0.07351-0.0124 = 0.0611 | 0.0008x100/0.07351 = 16.8685 | 16.8685-11.1005= 5.7679 | 5.7679x100/11.1005 = 51.9607 |

Table XI: *Effect of Blank solution on COM formation*

| Sl.No. | Solvent | BR | $Ca^{2+}$ in solution(g) | $Ca^{2+}$ in precipitate(g) | % Inhibition |
|---|---|---|---|---|---|
| 1 | Water | 1.2 | 0.0008x1.2 =0.00096 | 0.07351- 0.00096 =0.0726 | 0.00096x100/0.07351 =1.3059 |
| 2 | Urine | 2.5 | 0.0008x2.5 =0.0020 | 0.07351- 0.00020 =0.07151 | 0.0020x100/0.07351 =2.7207 |

Table XII*: Effect of PE on COM formation in aqueous medium*

| Sl. No. | Plant name | Inhibitors 0.1% | $Ca^{2+}$ in solution(g) | $Ca^{2+}$ in precipitate(g) | % of Inhibition | Diff. in % of inhibition between sample and blank | Relative % of inhibition |
|---|---|---|---|---|---|---|---|
| 1 | *Pratia nummularia* Kurz | Crude BR=2.8 | 0.0008x2.8 =0.00224 | 0.07351-0.00224 =0.07127 | 0.00224x100/0.07351 =3.0472 | 3.0472-1.3821 = 1.6651 | 1.6651x100/1.3821 =120.4761 |
| 2 | *Cyperus rotundus*Linn | Crude BR = 2.4 | 0.0008x2.4 =0.0192 | 0.07351-0.00192 =0.07159 | 0.0192x100/0.07351 =2.6119 | 2.6119-1.3059 =1.3060 | 1.3060x100/1.3059 =100.0077 |
| 3 | *Citrus latipes* | Crude BR=1.9 | 0.0008x1.9 =0.00152 | 0.07351-0.00152 =0.07199 | 0.0152x100/0.07351 =2.0677 | 2.0677-1.3059 =0.7618 | 0.7618x100/1.3059 =58.3352 |

Table XIII: *Effect of PE on COM formation in urinary medium*

| Sl. No. | Plant name | Inhibitors 0.1% | $Ca^{2+}$ in solution(g) | $Ca^{2+}$ in precipitate(g) | % of Inhibition | Diff. in % of inhibition between sample and blank | Relative % of inhibition |
|---|---|---|---|---|---|---|---|
| 1 | *Pratia nummularia* Kurz | Crude BR=5.3 | 0.0008x5.3 =0.00424 | 0.07351-0.00424 =0.0693 | 0.00424/100/.07351 =5.7679 | 5.7679-3.4009 =2.3670 | 2.3670x100/0.3.4009 =69.5992 |
| 2 | *Cyperus rotundus* | Crude | 0.0008x6.2 | 0.07351- | 0.00496x100/0.07351 | 6.7474-2.7207 | 4.0267x100/2.7207 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Linn | BR=6.2 | =0.00496 | 0.00496 =0.0686 | =6.7474 | =4.0267 | =148.0023 |
| 3 | *Citrus latipes* | Crude BR=7.0 | 0.0008x7.0 =0.0056 | 0.07351-0.0056 =0.0679 | 0.0056x100/0.07351 =7.6506 | 7.6506-2.7207 =4.9293 | 4.9293x100/2.7207 =181.1997 |

## III. RESULT AND DISCUSSION

From the above inhibition experiment, it is clear that the activity of inhibition was greater than blank aqueous and blank urine showing the potential effectiveness of the plant extract in CP and COM formation. It is seen that the inhibitory effect in the mineralization of stone forming chemicals in blank urine were more than aqueous medium. This shows that there may be some natural inhibitor in urine.

Out of the three plants, *Cyperus rotundus* Linn has the highest inhibitory effect in the mineralization of CP in aqueous medium while *Citrus latipes*(Heiribob, figure1) has the highest inhibitory effect in the mineralization of CP in urinary medium. Further *Pratia nummularia* Kurz has the highest inhibitory effect in the mineralization of COM in aqueous medium while *Cyperus latipes* has the highest inhibition effect in the mineralization of COM in urinary medium. In the meantime we took Cystone, a Herbal drug, manufactured by Himalaya company. It is an Ayurvedic properietory medicine for kidney stone cases. The chemoinhibitory of cystone and the three plants in aqueous and urinary media for CP and COM is shown in Table 14.In the comparative study Cystone has more inhibitory capacity for CP than the three plants both in aqueous and urinary media. But all the three plants have more inhibitory effect for COM both in aqueous and urinary media except the plant *Citrus Latipes* whose inhibitory power for COM in aqueous medium greater by Cystone.

Table XIV: *Comparison of Chemoinhibitory effect of Cystone and the three plants*

| Sl.No. | Name of drug & Plant | Type of stone | Aqueous medium | | Urinary medium | |
|---|---|---|---|---|---|---|
| | | | % Inhibitor | %Relative Inhibition | %Inhibitor | %Relative inhibition |
| 1 | Cystone | CP | 31.6485 | -ve | 47.3400 | -ve |
| | | COM | 1.6324 | 25.0000 | 4.4619 | 68.8595 |
| 2 | *Pratia nummularia* Kurz | CP | 9.6858 | 8.5411 | 11.2910 | 1.7161 |
| | | COM | 3.0472 | 120.4716 | 5.7679 | 69.5992 |
| 3 | *Cyperus rotundus* Linn | CP | 10.7740 | 20.7319 | 13.6036 | 22.5494 |
| | | COM | 2.6119 | 100.0077 | 6.7474 | 148.0023 |
| 4 | *Citrus latipes* | CP | 9.7946 | 9.7558 | 16.8685 | 51.9607 |
| | | COM | 2.0677 | 58.3352 | 7.6506 | 181.1997 |

Fig. I*: Citrus latipes(Heiribob)*

## IV. CONCLUSION

*Pratia nummularia* Kurz(Kihomman), *Cyperus rotundus* Linn(Sembang kaothum) and *Citrus latipes*(Heiribob) have high activity to inhibit the stone formation which may be either CP or COM. However, these plants are effective in controlling COM stone formation than that of CP formation. If such plants are fed to the kidney stone patients, it can reduce the size of kidney stone to some extent. However we are still continuing our investigation with other medicinal plants and will find out which plants has the highest dissolving power of kidney stone and which chemical compounds present in these plants are actually involved in the process. And also further investigation are required to determine exact doses and its side effects to the human trial.

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