

Comparison of Antiurolithiatic Property of *Orthosiphon spiralis*, *Hedychium marginatum*, *Thunbergia alata* and Cystone: A Herbal Drug

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ABSTRACT

Kidney is one of the vital parts of the human body. Malfunction of kidney is due to blockage of stone in the urinary tract and urinary infection. Peoples are mainly focused on the treatment of kidney stone by using medicinal plants. Patients suffering from kidney stone may be treated by Lithotripsy, Ureteroscopy, open or Laparoscopy. Such treatment is costly and painful. Now-a- days medicinal plants are mainly used for such treatment because these plants are less side effect and more economic. In the present study, the inhibitory potency of crude extracts of *Orthosiphon spiralis*, *Hedychium marginatum* and *Thunbergia alata* in methanol were evaluated on the formation of calcium phosphate (CP) and on the growth of calcium oxalate monohydrate (COM) crystals in vitro. Also, comparison of antiurolithiatic property of these plants and Cystone, a herbal drug, is studied. Results show that Cystone is effective in the inhibition of CP stone both in aqueous and urinary media while *Orthosiphon spiralis* has the highest inhibitory effect for COM both in aqueous and urinary media in vitro.

Key words: Lithotripsy, Kidney stone, Herbal drug, Inhibition, Antilithiatic, Ureteroscopy

INTRODUCTION

Formation of kidney stone is a serious and debilitating problem throughout the world. The incidence of kidney stone has increased in the last six decades in association with living life style and economic development. Most calculi in the urinary system is due to the formation of COM and CP. [1,2] The recurrence of urolithiasis represent a serious problem, as patients who have formed stone are more likely to form another, and thus stone formation is highly recommended. The kidney stone may be treated with new technology like Lithotripsy, exposure to shock wave, surgical operation etc. These are mainly associated with several adverse effects, including renal injury, decrease renal function and more important one is the increase of stone recurrence. [3] Therefore, it is worthwhile to look for an alternative way for the treatment of kidney stone i.e. antiurolithiasis by using medicinal plants.

[4,5] Treatment with medicinal plants shows less side effect and more economic. [6] In this respect, many plants have been used to treat kidney stone and shown to be effective among the medicinal plants. In our vitro study, the plant extracts (PE) are used in urine to its therapeutic as preventive agent (Antiurolithiasis) hindering the formation of COM and CP crystals. Different experimental procedures have been proposed using synthetic, diluted or natural supersaturated aqueous solution of urine. [7] Crystallization can be triggered by adding calcium, oxalates or phosphates to the reaction medium. Crystallization can also be done by changing the P^H of the substance having P^H dependent solubility. [8] Our aim is the avoidance of urolithiasis by preventing nucleation, growth and aggregation of CP and COM in the urinary tract or kidney by using medicinal plants and search more efficient and novel inhibitor of plants source. We are still

continuing our study to find out the chemical compounds actually involved in the inhibitory activity in these medicinal plants.

names, local names and the parts of plants used.

MATERIALS AND METHODS

Table 1: Medicinal plants with scientific and local names and plant used parts.

SL.No.	Scientific name	Local Name	Part of plant used
1	<i>Orthosiphon spiralis</i>	Leikhaman	Flower
2	<i>Hedychium marginatum</i>	Takheillei angangba	Root
3	<i>Jhubergia alanta</i>	Lilha	Leaf

Many Medicinal plants were grown in the different parts of Manipur. Healthy plants (*Orthosiphon spiralis* (figure 1), [9] *Hedychium marginatum* [9] and *Thunbergia alata*, [9] whose flower, root and leaf) were collected from the different district of Manipur. The parts of the plants were washed, dried, chopped and powdered. The dried powder parts of the plants were soaked in 50% methanol in a soxhlet extractor under hot condition. The plant extracts (PE) were distilled under reduced pressure using Rotary Vacuum Evaporator (RII) to produce crude mass which further spread in Petridis and dried in the desiccators. Table 1 gives the scientific



Figure 1: *Orthosiphon spiralis* (Leishman)

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. Urine was just required as a solvent to mimic the natural solvent system. In our study, we always used fresh urine. Water contents of the three plants were determined and are shown in Table 2.

Table2: 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	<i>Orthosiphon spiralis</i>	Flower	11.7330	4.8680	6.8650
2	<i>Hedychium marginatum</i>	Root	9.2220	1.4480	7.7704
3	<i>Jhubergia alanta</i>	Leaf	1.6110	0.2020	1.4090

Chemoinhibitory experiments of the plants including blank reading both in aqueous and urinary media were done. The experimental outcomes are shown in Tables (3to7).

Table 3: Inhibition experiment for CP (Blank)

Sl.No.	Water – Blank for CP				Urine – Blank CP			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	6.1	6.1		0	10.3	10.3	
2	0	6.0	6.0	6.0	0	10.2	10.2	10.2
3	0	6.0	6.0		0	10.2	10.2	

Table 4: Inhibition experiment for COM (Blank)

Sl.No.	Water – Blank for COM				Urine – Blank for COM			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	1.2	1.2		0	2.1	2.1	
2	0	1.2	1.2	1.2	0	2.0	2.0	2.0
3	0	1.2	1.2		0	2.0	2.0	

Table 5: Inhibition experiment for Orthosiphon spiralis

Sl.No.	Water – PE(0.1%) for CP				Urine – PE(0.1%) for CP			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	4.1	4.1		0	9.4	9.4	
2	0	4.0	4.0	4.0	0	9.3	9.3	9.3
3	0	4.0	4.0		0	9.3	9.3	
Water – PE(0.1%) for COM					Urine – PE(o.1%) for COM			
1	0	1.6	1.6		0	4.2	4.2	
2	0	1.5	1.5	1.5	0	4.1	4.1	4.1
3	0	1.5	1.5		0	4.1	4.1	

Table 6: Inhibition experiment for Hedychium marginatum

Sl.No.	Water – PE(0.1%) for CP				Urine – PE(0.1%) for CP			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	8.9	8.9		0	16.6	16.6	
2	0	8.8	8.8	8.8	0	16.5	16.5	16.5
3	0	8.8	8.8		0	16.5	16.5	
Water – PE(0.1%) for COM					Urine – PE(o.1%) for COM			
1	0	2.4	2.4		0	5.4	5.4	
2	0	2.3	2.3	2.3	0	5.3	5.3	5.3
3	0	2.3	2.3		0	5.3	5.3	

Table 7: Inhibition experiment for Jhubergia alanta

Sl.No.	Water – PE(0.1%) for CP				Urine – PE(0.1%) for CP			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	9.6	9.6		0	11.1	11.1	
2	0	9.5	9.5	9.5	0	11.0	11.0	11.0
3	0	9.5	9.5		0	11.0	11.0	
Water – PE(0.1%) for COM					Urine – PE(o.1%) for COM			
1	0	4.3	4.3		0	5.1	5.1	
2	0	4.2	4.2	4.2	0	5.0	5.0	5.0
3	0	4.2	4.2		0	5.0	5.0	

Chemoinhibition experiments were performed according to Rao TVRK. [10] 0.01M each of CaCl₂ and Na₃PO₄ were taken for CP crystallization. Similarly 0.01M each of CaCl₂ and Na₂OX were taken for CaOX 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(Tables 3 and 4). All the experiments were conducted at room temperature (25°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), EBT(1%) indicator and NH₃ – NH₄Cl as buffer(P^H-10). [11] 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 5 to 13). Inhibition efficiency was calculating by using the following equation.

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

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

where the total Ca²⁺ in the experiment equals the Ca²⁺ contents of 50ml CaCl₂ solution which was determined separately.

Table 8: Effect 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
Table 2	Urine	10.8	0.0008x12.8 = 0.00864	0.07351 – 0.00864 = 0.06487	0.00864x100/0.07351 =11.7535

Table 9: Effect of 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	<i>Orthosiphon spiralis</i>	Crude BR=4.0	0.0008x4.0 = 0.0032	0.07351-0.0032 =0.07031	0.0032x100/0.07351 = 4.3510	-ve	-ve
2	<i>Hedychium marginatum</i>	Crude BR=8.8	0.0008x8.8 = 0.0704	0.07351-0.0704 = 0.06647	0.00704x100/0.07351 =9.5769	9.5769-8.9239 =0.6530	0.6530x100/8.9239 =7.3174
3	<i>Jhubergia alanta</i>	Crude BR=9.5	0.0008x9.5 =0.0076	0.07351-0.0076 =0.06594	0.0076x100/0.07351 =10.3387	10.3387-8.9239 =1.4148	1.4148x100/8.9239 =15.8532

Table10: Effect of 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	<i>Orthosiphon spiralis</i>	Crude BR=9.3	0.0008x9.3 =0.00744	0.07351-0.00744 =0.0661	0.00744x100/0.07351 = 10.0061	10.0061-11.1005 =-ve	-ve
2	<i>Hedychium marginatum</i>	Crude BR=16.5	0.0008x16.5 =0.0132	0.07351-0.0132 =0.06031	0.0132x100/0.07351 = 17.9567	17.9567-11.1005 =6.8562	6.8562x100/11.1005 = 61.7648
3	<i>Thunbergia alata</i>	Crude BR=11	0.0008x11 = 0.0088	0.07351-0.0088 = 0.06471	0.0088x100/0.07351 = 11.9712	11.9712-11.1005 = 0.8707	0.8707x100/11.1005 = 7.8438

Table 11: Effect 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

Table12: Effect of 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	<i>Orthosiphon spiralis</i>	Crude BR=1.5	0.0008x1.5 =0.0012	0.07351-0.0012 =0.07231	0.0012x100/0.07351 =1.6324	1.6324-1.3821 = 0.2503	0.2503x100 1.3821 =18.1101
2	<i>Hedychium marginatum</i>	Crude BR = 2.3	0.0008x2.3 =0.0184	0.07351-0.0184 =0.0717	0.0184x100/0.07351 =2.5031	2.5031-1.3059 =1.1972	1.1972x100/1.3059 =91.6762
3	<i>Thunbergia alata</i>	Crude BR=4.2	0.0008x4.2 =0.00336	0.07351-0.00336 =0.07015	0.00336x100/0.07351 =4.5708	4.5708-1.3059 =3.2619	3.2619x100/1.3059 =250.0110

Table13: Effect of 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	<i>Orthosiphon spiralis</i>	Crude BR=4.1	0.0008x4.1 =0.00328	0.07351-0.00328 =0.07023	0.00328x100/0.07351 =4.4620	4.4620-3.4009 =1.0611	1.0611x100/3.4009 =31.1999
2	<i>Hedychium marginatum</i>	Crude BR=5.3	0.0008x5.3 =0.00424	0.07351-0.00424 =0.0693	0.00424x100/0.07351 =5.7679	5.7679-2.7207 =3.0472	3.0472x100/2.7207 =112.0006
3	<i>Thunbergia alata</i>	Crude BR=5.0	0.0008x5.0 =0.0040	0.07351-0.0040 =0.0695	0.0040x100/0.07351 =5.4414	5.4414-2.7207 =2.7207	2.7207x100/2.7207 =100.0000

RESULTS AND DISCUSSION

The chemoinhibitory experiments showed that the inhibitory power of the plants were more than blank aqueous and

blank urine. Hence it is clear that the plant extracts have greater inhibitory power for CP and COM stone formation. Further, it is shown that the inhibitory effects in the

mineralization of stone forming chemicals in blank were more than that in aqueous medium. Thus, it is learned that there may be some natural inhibitor in urine i.e. plant extract. Among the three plants *Thunbergia alata* (Lilha) has the highest inhibitory effect for CP and COM stone formation in the aqueous medium while *Hedychium marginatum* (Takhellei angangba) shows the

highest inhibitory effect for CP and COM stones formation in the urinary medium.

We, further, are continuing the inhibitory experiment with Cystone, a herbal drug, manufactured by the Himalayan Company and compared the inhibitory effects with the three plants (shown in Table 14).

Table 14: 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	64.0019
2	<i>Orthosiphon spiralis</i>	CP	4.3510	-ve	10.0067	-ve
		COM	1.6324	18.1101	4.4619	64.0019
3	<i>Hedychium marginatum</i>	CP	9.5769	7.3174	17.9567	61.7648
		COM	2.5031	91.6762	5.7679	112.0006
	<i>Thunbergia alata</i>	CP	10.3387	15.8532	11.9712	7.8438
		COM	4.5708	250.0110	5.4414	100.0000

From the Table 14, it is seen that the chemoinhibitory effect for CP stone is dominated by Cystone, but it is less effective for COM stone as compared to the three plants i.e. these medicinal plants have greater inhibitory power for COM stone than Cystone, a herbal drug.

CONCLUSION

The objective of our investigation is to find out which plants have the highest inhibitory effect for CP and COM stone formation. The plant extracts of *Orthosiphon spiralis*, *Hedychium marginatum* and *Thunbergia alata* show antiurolithiatic (i.e., chemoinhibitory effect in stone formation) property both in aqueous and urinary media for CP and CaOX stone. These plants are more effective in controlling CaOX stone formation than that of CP stone formation. It can further concluded that the plant extracts (flower, root and leaf)of the above three plants are less effective than that of Cystone for inhibition of CP stone formation. But these extracts are more effective than Cystone in the inhibition of CaOX stone formation. If such plant extracts are fed to the kidney stone patients, we can monitor the decreasing the sizes of the kidney stone. We are still continuing our experiment with

other medicinal plants and which chemical compounds present in these plants are actually involved in the digestion of the kidney stone.

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