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Study of the Effect of Some Ethnomedicinal Plants in the Treatment of Kidney Stones Specially Calcium Oxalate Through Chemoinhibition Experiment *in vitro* Condition

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ABSTRACT

Many novel compounds had obtained from the plants having medicinal property. Among the medicinal plants, those having antiurolithiatic properties are important and are very effective in controlling and curing of stone formation in the urinary tract. The inhibitory effect of aqueous methanolic extract of traditionally used medicinal plants were studied on the formation of calcium oxalate (CaOX) stone in aqueous as well as in urinary medium. The inhibitory effects were presented as the percentage of inhibition or the degree of inhibition as compared to blank urine. The inhibition in stone formation is due to its capacity of increasing the solubility product of its constituents and is determined through chemoinhibition experiment. The antiurolithiatic properties of different medicinal plants were studied and presented in decreasing order in the present work. The finding give support the effectiveness of many medicinal plants against the urolithiatic cases specially in CaOX formation as used in the folklore medicine.

***Key words*:** Novel, Antiurolithiatic, Inhibitory, Chemoinhibition, Urinary, Folklore

INTRODUCTION

The stone cases faced by peoples in the worldwide is a serious problem. Among it, the kidney stone cases are treatable by traditionally methods using the locally available medicinal plants. Manipur (India), a small north eastern state of India has a rich traditional knowledge in treating many ailments and diseases by using medicinal plants. The state itself has a rich resource of many medicinal plants having medicinal properties because of its location in the biodiversity hot spots of the world and its microclimatic conditions. The present work reports how far the traditionally used medicinal plants has effectiveness in controlling and curing the stone cases in the kidney and its tract under in vitro condition. The work has focused and strictly maintained the traditional method in selecting the part of the plant used.

The kidney stone case is a serious clinical condition which may lead to major causes for acute and chronic renal failure. It is mainly common in man than woman but rare in children [1]. In 1-5% of the population, the root cause of kidney stone formation is multifactorial and some of them may be dehydration, urinary tract infection by microorganism, alkaline, P^H and abnormality in calcium phosphate(CP) metabolisms[2,3]. A low level of natural inhibitors of calculogenesis in urine may also another factor that tends to urolithiasis.

The main constituents of stones from the kidney and its tract are calcium containing

stones , comprising about 75% of all urinary calculi. It may be in the form of pure CaOX (50%) or CP (5%) and a mixture of both(45%)[2] . The urinary stone begins its growth from the occurrence of nuclei through nucleation and then becomes aggregated. The supersaturation of urine leads to the formation of a nuclei and it depends on urinary P^H, ionic strength , solute concentration and infection by pathogenic microbes[4].

In the present study, the aqueous methanolic extract of the part of the plant was used to study the chemoinhibitory effects on the mineralization of calcium oxalate stone both in urinary and aqueous media under in vitro conditions. In the work, natural diluted solution of urine are taken and the effect of plant extract are observed in the nucleation of CaOX . The aim of the study was to search which plant is more effective in controlling or curing of CaOX formation. It will give support to use medicinal plants which is less toxic and more cost effective . The ongoing research work will open a gateway to explore novel compounds from the natural resources.

MATERIALS AND METHODS

Collection of plant sample and Preparation of extract: Ten traditional medicinal plants used in the kidney stones were selected for analysis(Table 1).

Table 1: Plants, their scientific names [5] and parts used in analysis

Sl.No.	Name of Plant	Local name & Part of plant used
1	*Piper nigrum*	Gul & fruit
2	*Aegle marmelos*	Heirikhagok & fruit
3	*Plantago major*	Yempat & leave
4	*Curcuma lorga*	Turmeric & rhizome
5	*Ficus carico*	Theibong & fruit
6	*Allium odorosum*	Maroi nakupi & leave
7	*Mentha arvensis* Linn	Nungsihidak & leave
8	*Centella asiatica*	Peruk & whole plant
9	*Celtris australis* Linn	Heikreng & fruit
10	*Cyprus rotundus*	Sembang kouthum & whole plant

The part of the plants to be used either leave, stem or fruit[6], was collected from the market of Imphal, Manipur or from local if available. They were washed and the healthy parts were taken and air dried in shady place. The aqueous extract was prepared by extracting with methanolic distilled water(50% MeOH) using soxhlet apparatus. The aqueous extract was distilled under reduced pressure using Rotary vacuum evaporator to crude mass. The crude mass thus obtained was spread in a glass sieve for 5 days under open air to remove residual solvent when the solid crude mass was obtained.

Collection of Urine

Urine is required just as solvent to mimic the natural solvent system. It was collected from a healthy male(30yrs) who does not have any stone cases, in a sterilized container and camphor is added as preservative. The freshly collected urine was always used in the experiment and collection was done during 9 – 10 am before taking lunch by the individual.

Inhibition experiment :

An experimental model was set up according to T.V.R.K. Rao and et al[7]. 0.01M each of calcium chloride and sodium oxalate were taken for CaOX crystallization . 50ml of plant extract(0.02% of crude) in urine was taken as inhibitor solution. Simultaneous blank expt. with urine in place of inhibitor solution were also carried out for evaluating the inhibition efficiency(IE) of inhibitors compared to urine.

All experiments were conducted at room temperature (25^{o}C). Calcium content of the centrifugate left after stone had formed, is determined by complexometric method using standard disodium EDTA solution (0.01M)[7,8]while calculating the calcium content of the centrifugate, a titre value of EDTA vs corresponding total inhibitor

solution was deduced from the total titre values was calculated using the formula

Inhibition efficiency (i.e.% inhibition)= $\frac{Ca^{2+} \text{ in centrifugate}}{\text{Total } Ca^{2+} \text{ in expt}}$ x 100 ----------(1)

Where the total Ca^{2+} in the expt. equals the Ca^{2+} contents of 50 ml of 0.01 $CaCl_2$ solution, which was determined separately.
From the % inhibition values, the increase of inhibition efficiency(IE) over blank(urine) was calculated out, % increase of IE of inhibitors relative to blank were also calculated out using the formula
% increase of inhibition relative to blank = $\frac{\text{Increase of \% inhibition over blank}}{\text{\% inhibition by blank}}$ ----------(2)

In the complexometric titration of centrifugate with disodium EDTA, P^H was maintained at 10 using NH_4Cl-NH_3 buffer and the mixture of EBT & KNO_3 was used as indicator of end point of titrap tion(wine red color changes to blue)[7,8].

EXPERIMENTAL

Table 2: Inhibition Experiment in Aqueous Medium

Sl.No.	Name of Plant	Ca^{2+} in Soln(g)	Ca^{2+} in ppt(g)	% Inhibition	Increase of Inhibition over blank	%Increase of Inhibition
1	*Piper nigrum*	0.0012	0.0723	1.6324	0.3265	25.0000
2	*Aegle marmeplos*	0.0029	0.0706	3.9178	2.6119	260.5749
3	*Plantago major*	0.0035	0.0700	4.7885	4.4825	266.6666
4	*Curcuma lorga*	0.0020	0.0715	2.7207	1.4148	108.3404
5	*Ficus carico*	0.0029	0.0447	8.2051	6.8992	528.3120
6	*Allium odorosum*	0.0010	0.0726	1.3060	-	-
7	*Mentha arvensis* Linn	0.0010	0.0726	1.3060	-	-
8	*Centella asiatica*	0.0012	0.0723	1.6324	0.3265	25.0000
9	*Celtris australis* Linn	0.0036	0.0699	4.8973	3.5914	275.0000
10	*Cyprus rotundus*	0.0099	0.0716	2.6119	1.3060	100.0077

Table 3: Inhibition Experiment in Urinary Medium

Sl.No.	Name of Plant	Ca^{2+} in Soln(g)	Ca^{2+} in ppt(g)	% Inhibition	Increase of Inhibition over blank	%Increase of Inhibition
1	*Piper nigrum*	0.0089	0.0654	10.9917	7.5909	223.2034
2	*Aegle marmelos*	0.0040	0.0696	5.4958	2.7751	102.0013
3	*Plantago major*	0.0080	0.0655	10.8828	8.1621	300.0000
4	*Curcuma lorga*	0.0080	0.0655	10.8828	8.1621	300.0000
5	*Ficus carico*	0.0028	0.0707	3.8090	1.0883	40.0009
6	*Allium odorosum*	0.0030	0.0706	4.0267	1.3060	48.0034
7	*Mentha arvensis* Linn	0.0034	0.0702	4.5708	1.8502	68.0040
8	*Centella asiatica*	0.0046	0.0887	6.3121	3.5914	132.0005
9	*Celtris australis* Linn	0.0057	0.0678	7.7268	5.0061	184.0005
10	*Cyprus rotundus*	0.0050	0.0686	6.7474	4.0267	148.0007

RESULT AND DISCUSSION

The effects of the 0.1% of the crude mass of the plants(Table 1) were studied on the nucleation and growth of CaOX in aqueous as well as in urinary medium. For every set of experiments, blank experiments, blank experiments were always performed in both media we had performed the inhibition experiments of a particular plant in the medium at the same day to avoid any discrepancies . In the inhibition experiment, the % inhibition in the presence of plant extract in CaOX formation. Urinary stone is the result of supersaturation of urine with certain urinary salts such as CaOX or calcium phosphate(CP)[9]. In order to access the inhibitory effects in the mineralization of stone forming chemicals, blank urine was also studied and found that it showed some inhibitory effect which may be due to presence some natural inhibitors[10,11].

Using the relation (1), the percentage inhibition or IE of the different plant extracts were calculated (Table 2). It was found that *Ficus carico* show the highest IE (8.2051%) while *Allium odorosum* and Mentha *arvensis* Linn show the lowest IE (1.3059%). *Piper nigrum* and *Centella asiatica* have the same IE (1.6324%). *Celtris australis* Linn (IE = 4.8972) and *Plantago major* (IE = 4.7884) also have high IE over the remaining plants studied. The following chart represent the decreasing order of IE 0.1% plant extract in aqueous medium (Fig.1). *Fiscus carico*(8.2051%) > *Celtris australis* Linn(4.8972%)> *Plantago major*(4.7884%)>*Aeglemarmelos*(3.9178%) >*Curcumalorga*(2.7207%)>*Cyprusrotundus* (2.6118%)>*Centellaasiatic*(1.6324%); *Piper nigrum*(1.6324%)>*Alliumodorosum*(1.3059 %); *Mentha arvensis* Linn(1.3059%).

The percentage inhibition or inhibition efficiency in urinary medium were also calculated using the relation(1). Surprisingly it was found that the inhibition efficiency were far higher in urinary medium than the aqueous medium. Also, it was found that the *Piper nigrum*(IE=10.9917) showed the highest IE and *Ficus carico*(IE=3.8090) the lowest. The following bar chart also showed the decreasing order of IE of the plant extracts (0.1%w/v) (Fig.2) *Pipernigrum*(10.9917%)>*Curcumalorga*(10. 882850)> *Celtris australis* Linn(7.7268%)> *Cyprusrotundus*(6.7373%)>*Centellaasiatica* (6.3120%)>*Plantagomajor*(5.6046%)>*Aegl emarmelos*(5.5708%)>*Menthgaarvensis*Lin n(4.5708%)> *Allium odorosum*(4.0266%)> *Ficus carico*(3.8090%).

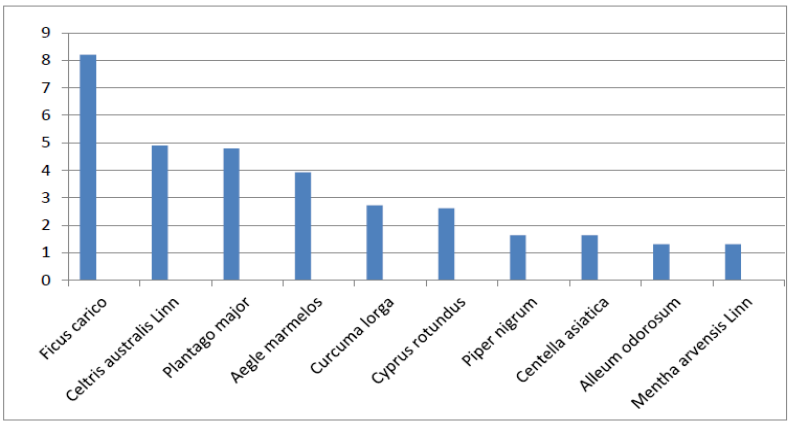

Figure 1: Comparison of inhibition efficiencies of plants in aqueous medium

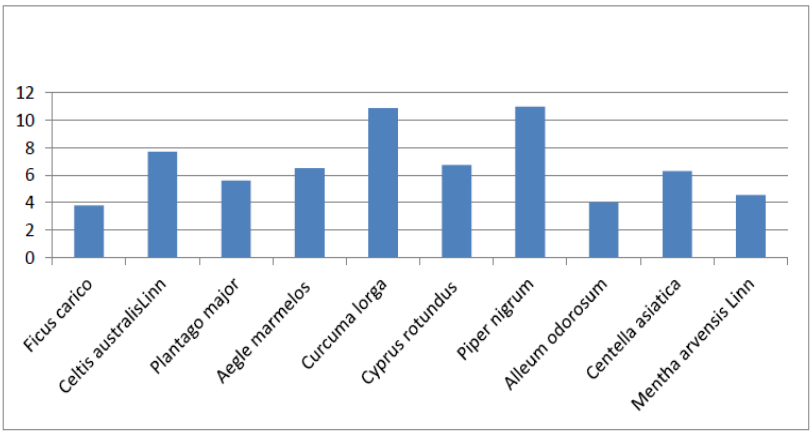

Figure 2: Comparison of inhibition efficiencies of plants in urinary medium

CONCLUSION

The main aim of the research work is to evaluate the effectiveness of the traditionally
used medicinal plant used in treating stone cases in urinary tract. Through our research work, it was found that all of them show effectiveness in the inhibition of nucleation and precipitation of CaOX in both aqueous and urinary media. The plant *Piper nigrum* extract shows the highest inhibition efficiency in urinary medium though it gives lowest efficiency in aqueous medium. Therefore, it can be concluded that we can get more effectiveness by using those plants which show more inhibition efficiency in urinary medium. We are still continuing our research work in antimicrobial activity as well as in chemical composition of the plant extract.

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