

Comparative Study of the Antiurolithiatic Activity of Plant Hydroalcoholic Extract of *Argemone mexicana*, *Cissus javana* DC and *Garcinia pendunculata*

O. Ibopishak Singh¹, A. Bimola Devi²

Chemistry Department, Modern College, Imphal East - 795005, Manipur, India

Abstract: Urinary stones are one of the oldest and the most common afflictions in humans. These diseases have suffered since the earliest records of civilization. 10% of men and 3% of women have a stone during their adult lives while the chances of urinary stone are less in children. Calcium containing stones are the most common comprising of about 75% of all urinary calculi, which may be in the form of pure calcium oxalate (50%) or calcium phosphate (5%) or a mixture of both (45%). A number of medicinal plants have been mentioned in the Indian ayurvedic system which plays a vital role in the inhibition of stones. Traditional and folklore medicines play important role in health services around the globe. About three quarter of the world's population relies on plants and plant extracts for health care. In the present study, the inhibitory potency of crude extracts of *Argemone mexicana*, *Cissus javana* DC and *Garcinia pendunculata* in methanol were evaluated on the formation of calcium phosphate and on the growth of calcium oxalate monohydrate (COM) crystals in vitro. Results obtained indicated that *Argemone mexicana* has the highest inhibition the formation of CP in the urinary medium while the *Garcinia pendunculata* has the highest inhibition the formation of COM in the urinary medium in vitro.

Keywords: Folklore medicines, Inhibitors, Urinary calculi, Antiurolithiasis, Allopathic drugs

1. Introduction

The kidney stones are known as being a painful health problem. It is a serious chemical condition which may lead to major causes for acute and chronic renal failure. The kidney stone formation is influenced by the dietary habits. A diet low in calcium is more influencing in stone formation than a diet high in calcium. Also ingestion of diet containing high amount of oxalate enhances stone formation than a diet containing more calcium. That is, oxalate is a very stone promoter of calcium oxalate precipitation, about 15 times stronger than calcium¹. Because of the side effects of the allopathic drugs for the treatment of kidney stone patients and their relatives seek herbal medicines as a prospective treatment for this condition i.e. according to herbal scientists report herbal medicines have the least side effects. Patients between 31 to 51 years suffered the most. In this paper we are mainly focusing on the treatment of plant extracts of *Argemone mexicana*, *Cissus javana* DC and *Garcinia pendunculata* with the kidney stones (collected) in vitro. Further, the present research of kidney treatment will be useful for the general public and also for the conservation of helpful medicinal plants in home garden and the public places like Eco Park, recreational parks, etc.

The kidneys are one of the most vital parts of human body^{2, 3, 4}. Malfunction of the kidney due to the blockage of urine by stone causes unbearable pain⁵. It is a serious health problem for one and all. Now-a-days many people seek herbal medicine instead of allopathic drugs. The traditional healers (herbalists) expressed that the lower abdominal pain is due to the block of urinary tract by the stone and the symptoms are started with incessant vomiting⁶. According to the scientists and researchers, there are four kinds of kidney stones⁷ viz. calcium, cystine, struvite and uric acid.

Calcium is the most common form of kidney stones while cystine is the least common variety.

Treatment of kidney stone problem by traditional healers (herbalists) is still going on among the local practitioners like meiteis, muslims⁸ and tribals. Shri L.Nabakishore Singh (a local healer), a Padmashree recipient, is still giving the treatment of kidney stone with herbs. In the present investigation, COM and CP were formed in the artificial urine technique. The effects of hydromethanolic extracts of *Argemone mexicana*, *Cissus javana* DC and *Garcinia pendunculata* were studied on the growth and inhibition of COM and CP in vitro. Further we confirmed the inhibitory effect of the growth of COM and CP by the plant extracts as comparing the dissolution of kidney stones (collected from patients) by the plant extracts. In the in vitro study, we show that extracts from the plants are used in urine owing to its therapeutic potential as a preventive agent by hindering the formation of COM and CP crystals.

2. Methods

Collection of the healthy plants (leaves and fruits) of *Argemone mexicana*, *Cissus javana* DC and *Garcinia pendunculata* (Table 1) were done. The herbarium of the plants is already reported. The parts of the plants were washed, dried, chopped and powdered. In the mean time kidney stones were collected from the Professor S.Rajendro Singh, Department of Orthopedics, RIMS, Lamphel, Manipur, India. The dried powdered leaves and fruits of the three plants were soaked in 50% aqueous methanol in a soxhlet extractor under hot condition. The extracts were distilled under reduced pressure using Rotary Vacuum Evaporator (R11) to produce crude mass which further spread in Petridish and kept in desiccators.

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

S. No	Plant species	Local name	Part used
1	<i>Argemone mexicana</i>	Khomthokpee	Leaves
2	<i>Cissus javana</i> DC	Kongouyen laba	Leaves
3	<i>Garcinia pendunculata</i>	Heibung	Fruits

2.1. Collection of urine

Urine is required just as solvent to mimic the natural solvent system. It 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. The freshly collected urine was always used in the experiment.

2.2. Experimental

Water contents of the plants were determined and are shown in Table 2.

Table 2: Water contents

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>Argemone mexicana</i>	Leaves	1.0760	0.3710	0.7050
2	<i>Cissus javana</i> DC	Leaves	1.0000	0.7210	0.2790
3	<i>Garcinia pendunculata</i>	Fruits	3.9210	0.6400	3.2810

Inhibitory experiments of the plants including blank readings both in aqueous and urinary media were performed. The experimental findings are shown in the Tables (3 to 7).

Table 3: Inhibition Experiment for CP (Blank)

Sl. No.	Water - Blank for CP				Urine - Blank for CP			
	IR (ml)	FR (ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR9M(ml)	Diff.(ml)	Mean
1.	0	6.3	6.3		0	10.2	10.2	
2.	0	6.0	6.0	6.0	0	10.2	10.2	10.2
3.	0	6.0	6.0		0	10.1	10.1	

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	2.3	2.3	
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 *Argemone mexicana*

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	8.5	8.5		0	15.6	16.5	
2	0	8.6	8.6	8.5	0	15.5	16.5	15.5
3	0	8.5	8.5		0	15.5	16.7	
Sl. No.	Water - PE(0.1%) for COM				Urine - PE(0.1%) for COM			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	2.1	2.1		0	11.8	11.8	
2	0	2	2	2	0	11.7	11.7	11.7
3	0	2	2		0	11.7	11.7	

Table 6: Inhibition Experiment for *Cissus javana* DC

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	8.5	8.5		0	9.8	9.8	
2	0	8.5	8.5	8.5	0	9.7	9.7	9.76
3	0	8.5	8.5		0	9.8	9.8	
Sl.No.	Water - PE(0.1%) for COM				Urine - PE(0.1%) for COM			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	0.9	0.9		0	4.6	4.6	
2	0	0.9	0.9	0.9	0	4.6	4.6	4.6
3	0	0.9	0.9		0	4.6	4.6	

Table 7: Inhibition Experiment for *Garcinia pendunculata*

Water – PE(0.1%) for CP					Urine – PE(0.1%) for CP			
Sl.No.	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	9.3	9.3		0	11.9	11.9	
2	0	9.3	9.3	9.3	0	11.7	11.7	11.8
3	0	9.3	9.3		0	11.8	11.8	
Water-PE(0.1%) for COM					Urine – PE(0.1%) for COM			
Sl.No.	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR (ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	1.7	1.7		0	4.6	4.6	
2	0	1.6	1.6	1.7	0	4.8	4.8	4.8
3	0	1.7	1.7		0	4.8	4.8	

Inhibition experiments were performed according to T.V.R.K. Rao et al⁹. 0.01 M each of CaCl₂ and Na₃PO₄ were taken for CP crystallization. Similarly 0.01 M each of CaCl₂ and Na₂Ox were taken for CaOX crystallization. 50 ml 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 for evaluating the inhibition efficiency of inhibitors compared to water or urine (Table 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 volumes. 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)¹⁰, EBT(1%)

indicator and NH₃-NH₄Cl 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 values (equivalent to centrifugate)(Table 5 to 13). Inhibition efficiency was calculated by using the following equation,
 Inhibition efficiency (i.e. % Inhibition)

$$= \frac{\text{Ca}^{2+} \text{ in centrifugate}}{\text{Total Ca}^{2+} \text{ in the experiment}}$$

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

where total Ca²⁺ in the experiment equals the Ca²⁺ contents of 50ml of 0.01M CaCl₂ solution which was determined separately.

Table 8: Effect on CP formation in Aqueous medium

Sl.No.	Plant name	Inhibitor 0.1%	Ca ²⁺ in solution(g)	Ca ²⁺ in ppt(g)	% of Inhibition	Diff. in % of Inhibition between sample & blank	Relative % inhibition
1	<i>Argemone mexicana</i>	Crude BR=8.8	0.0008x8.8 = 0.00704	0.07351-0.00704 = 0.06647	0.00709x 100/0.07351 = 9.5769	9.5769-8.9236 = 0.6530	0.6530x100/ 8.9239= 7.3194
2	<i>Cissus javana</i> DC	Crude BR= 8.5	0.0008x8.5 = 0.00680	0.07351-0.00680 = 0.06671	0.0068x 100/0.07351 = 9.2504	9.2504-8.9236 = 0.3365	0.3365x100/ 8.9239= 3.9076
3	<i>Garcinia pendunculata</i>	Crude BR=9.3	0.0008x9.3 = 0.00744	0.07351-0.00744 = 0.06607	0.00744x 100/0.07351 = 10.1211	10.1211- 9.9236 = 1.1971	1.1971x100/ 8.9239= 13.4147

Table 9: Effect on CP formation in Urinary medium

Sl. No.	Plant name	Inhibitor 0.1%	Ca ²⁺ in solution(g)	Ca ²⁺ in ppt(g)	% of Inhibition	Diff. in % of Inhibition between sample & blank	Relative % inhibition
1	<i>Argemone mexicana</i>	Crude BR=16.5	0.0008x16.5 = 0.0132	0.07351-0.0132 = 0.06031	0.0132x 100/0.07351 = 17.9567	17.9567- 11.1005 = 6.8562	6.8562x100/ 11.1005 = 61.7648
2	<i>Cissus javana</i> DC	Crude BR= 9.76	0.0008x9.76 = 0.007808	0.07351-0.007808 = 0.06570	0.007808x 100/0.07351 = 10.6217	- ve	- ve
3	<i>Garcinia pendunculata</i>	Crude BR=11.8	0.0008x11.8 = 0.0944	0.07351-0.0944 = 0.06407	0.0944x 100/0.07351 = 12.8418	12.8418 – 11.1005 = 1.7412	1.7412x100/ 11.1005 = 15.6858

Table 10: Effect on CP formation

S.No.	Solvent	BR	Ca ²⁺ in solution(g)	Ca ²⁺ in ppt(g)	% Inhibition
1	Water	10.2	0.0008x10.2= 0.00816	0.07351-0.00816=0.06535	0.00816x100/0.07351 = 11.1005
2	Urine	10.8	0.0008x10.8=0.00864	0.07351-0.00864=0.06487	0.00864x 100/0.07351=11.7535

Table 11: Effect on COM formation

Sl.No	Solvent	BR	Ca ²⁺ in solution(g)	Ca ²⁺ in ppt(g)	% Inhibition
1	Water	1.2	0.0008x1.2 = 0.00096	0.07351-0.00096 = 0.07255	0.00096x100/0.07351 = 1.3059
2	Urine	2.5	0.0008x2.5 = 0.0020	0.07351-0.0020 = 0.07151	0.0020x 100/0.07351 = 2.7207

Table 12: Effect on COM formation in Aqueous medium

Sl.No	Plant name	Inhibitor 0.1%	Ca ²⁺ in solution(g)	Ca ²⁺ in ppt(g)	% of Inhibition	Diff. in % of Inhibition between sample & blank	Relative % inhibition
1	<i>Argemone mexicana</i>	Crude BR=2.33	0.0008×2.33 = 0.001864	0.07351-0.001864 =0.071646	0.001864×100/0.07351 = 2.5357	2.5357- 1.3059 =1.2298	1.2298×100/1.3059= 94.1667
2	<i>Cissus javana</i> DC	Crude BR= 0.9	0.0008×0.9 =0.00072	0.07351-0.00072 =0.07279	0.00072×100/0.07351 = 0.9795	- ve	- ve
3	<i>Garcinia pendunculata</i>	Crude BR=4.5	0.0008×4.5 =0.0036	0.07351-0.0036 = 0.06991	0.0036×100/0.07351 = 4.8973	4.8973 – 1.3059 = 3.5913	3.5913×100/1.3059= 275.0000

Table 13: Effect on COM formation in Urinary medium

Sl. No	Plant name	Inhibitor 0.1%	Ca ²⁺ in solution(g)	Ca ²⁺ in ppt(g)	% of Inhibition	Diff. in % of Inhibition between sample & blank	Relative % inhibition
1	<i>Argemone mexicana</i>	Crude BR=5.3	0.0008×5.3 = 0.00424	0.07351-0.00424 =0.06927	0.00424×100/0.07351 = 5.7677	5.7677- 2.7207 = 3.0472	3.0472×100/2.7207= 112.0005
2	<i>Cissus javana</i> DC	Crude BR= 4.6	0.0008×4.6 =0.00368	0.07351-0.009368 =0.06983	0.00368×100/0.07351 = 5.006121	5.0061 - 2.7207 = 1.6252	1.6052×100/2.7207= 49.1203
3	<i>Garcinia pendunculata</i>	Crude BR=7.1	0.0008×7.1 =0.00568	0.07351-0.00568 = 0.06783	0.00568×100/0.07351 = 7.7268	7.7268 – 2.7207 = 5.0061	5.0061×100/2.7207= 184.0005

3. Discussions

In the inhibition experiment, the activity of inhibition in the presence of inhibitor was greater than blank aqueous and blank urine showing the potential effectiveness of the plant extract in CP and COM formation. The inhibitory effects in the mineralization of stone forming chemicals in blank urine were more than aqueous medium which showed that some natural inhibitors may be present in urine.

Out of the three plants, *Garcinia pendunculata* (Heibung)(Figure1) has the highest inhibitory effect in the mineralization of CP in aqueous medium while *Argemone*

mexicana(Khomthokpee) has highest inhibitory effect in the mineralization of CP in urinary medium. *Garcinia pendunculata* has the highest effect in the mineralization of COM both in aqueous and urinary media. We took *Cissus javana* DC (Kongouyen laba), which was frequently used by our traditional healers or herbalists, was treated with kidney stone (collected) in aqueous and urinary media. The experimental results are shown in Table 14. From the experimental results, it is clear that the plant has more inhibitory power in urinary medium than that in aqueous medium. It can easily dissolve the kidney stones (Figure 2 and 3).

Table 14: Treatment of *Cissus javana* DC with kidney stone

Sl No.	Solvent	% of Crude	Duration	Mass of Stone before treatment with PE	Mass of kidney stone after treatment with PE	Difference of mass of kidney
1	Water	0.1	4 hours	0.530 g	0.474 g	0.076 g
2	Urine	0.1	4 hours	0.849 g	0.740 g	0.079 g

4. Conclusion

These plants have potential to control stone formation which may be either CP or COM. Moreover, these plants are more effective in controlling CaOX stone formation than that of CP stone formation. If such a plant is fed to the kidney stone patient, it will help a lot. Similarly we are continuing our investigations with the other plants and will find out which plants has the highest stone dissolving power and which chemical compounds are actually involved in the process. There is possibility of using leaves and fruits of these plants as the therapeutic agent in antiurothiasis indicating its high amount of chemoinhibitory effects. Further investigations are required to determine exact doses and its side effects to the human trial.

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Figure 1: *Garcinia pedunculata* (local name Heibung)



Figure 2: Kidney stone without Plant extract(*Cissus javana* DC)



Figure 3: Kidney stone with Plant extract(*Cissus javana* DC)

Author Profile



Dr. Oinam Ibopishak Singh did M.Sc. (1988), Ph.D (2003). Name of University is M.Sc. (MU) & Ph.D (MU). He has published manuscripts in 4 International and 3 National journals. He has Experience in research for 21 years



Dr. Asem Bimola Devi did M.Sc. (1991), Ph.D(2009). Name of University: M.Sc. (MU) & Ph.D.(MU). He has published manuscripts in 4 International and 3 National journals. She has Experience in research for 19 years