

Evaluation of the Chemoinhibitory effects of Some Fruits found in Manipur on Kidney stone in *in vitro* and comparison of the effects with the herbal drug - Cystone

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Abstract

Now-a-days, most people are concerned about their health. Good health means almost free from all kind of diseases. Among these diseases, kidney stone disease is one of the most common ailments (3rd place) in the world. The most emerging technique for the treatment of kidney stone is the use of medicinal plants (leaf, fruit, flower, whole plant, etc.) because it is more economic and fewer side effect. The present research work is the study of the effects of locally available fruits of Manipur on kidney stones formation (mainly calcium oxalate stone formation) both in the aqueous and urinary media. Fruits are providing antioxidants, antibiotics, antiurolithiatic property, etc. The statistical correlation study report has said that when investigating the effect of fruit extract in aqueous and urinary media, a highly significant linear relationship was observed between Inhibition (0.1%) and the concentration of Ca^{2+} in solution (g). However, weak linear relations ($P > 0.05$) were observed between Inhibition (0.1%) and Ca^{2+} in precipitate (g), as well as between the concentration of Ca^{2+} in solution (g) and Ca^{2+} in precipitate (g). Among the fruits studied, *Celtis australis* has the highest inhibitory effect on calcium oxalate(COX) stone formation in aqueous medium while *Citrus latipes* has the highest inhibitory effect on COX stone formation in the urinary medium.

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Key words: Kidney stone disease, Medicinal plants, Antiurolithiatic property, calcium oxalate stone, statistical correlation, Inhibitory effect.

1. Introduction

Health is wealth. Good health is nothing but having no ailments. If a person is enjoying proper sleeping, appetite for taking food and proper functioning of sanitary practices, then the person is said to be healthy i.e. almost free from disease. Healthy food (nutritious food) and good exercise will help to achieve good health. Lack of proper diet and exercise lead to different kind of diseases like hypertension, cancer, kidney stone disease, (Dawson CH et al, 2012, Moe OW et al, 2006, Younis A et al, 2011, Han H et al, 2015, Mahmoud NAMRKNKSA et al, 2019 and Aleliign T et al, 2018) diabetes, hemophilia, etc.

Kidney stone disease (Coe FL et al, 2005 and Bihl G et al, 2001) is one of the most ailment in the world (3rd place) (Bhutani et al, 2008). Treatment of kidney stone disease can be done through open surgery, lithotripsy, allopathic drug, herbal drug like cystone, medicinal plants (Yadav RD, et al, 2011 and Kasole DM et al, 2017), etc. One of the most promising techniques is the treatment of medicinal plants (fruits) for the breakdown of kidney stones or blocking the formation of COX (Anamika et al, 2016, Muharren et al, 2019). Further medicinal plants (fruits) have fewer side effect than approved drugs on human health and treatment of medicinal plants (fruits) on any disease is more economy. From the time immemorial, it is known that fruits play an important role in the maintenance of good health of human body i.e. free from disease. Eating a variety of healthful fruits provide the body with nutrients and antioxidants including flavonoids that can boost overall health. Fruits are important source of essential vitamins and minerals. Further consumption of a lot of fruits can reduce a person's risk of developing heart diseases, cancer, inflammation and diabetes. Citrus fruits and berries may be especially powerful for preventing diseases. That is why most people is taking interest of the treatment of medicinal plants (fruits) on various diseases (Sheik et al, 2018). Kidney stones are mainly caused by a disruption in the equilibrium between solubility and precipitation of salts in the urinary system and kidneys. Excessive consumption of salts and animal proteins, as well as, inadequacies in chelating agents such as alkali foods, fiber and citrate are among the lifestyle habits and nutritional factors. Urinary stone precipitation or nucleation can also be aided by metabolic abnormalities such as hyperuricosuria (high amount of urinary uric acid excretion), hypercalciuria (excess of calcium in the urine), hyperoxaluria (elevated excretion of oxalate in urine), hypocitraturia (low urinary citrate excretion) and a history of gout (defective uric acid biotransformation) (Mohammed et al, 2022). Patient who experiences severe colic pain caused by kidney stones (Ankur C et al, 2020, Stamatelou KK et al, 2003 and Shu X et al, 2019)

are not often completely relieved by traditional pain killer medications. Additionally, severe urinary tract blockage, infection, hydronephrosis and significant urinary hemorrhage might commonly occur individuals with kidney stone. Now-a-days, due to the confirmed side effects of conventional drugs, the tendency of consuming of medicinal plants (fruits) has increased (Mahmoud et al, 2009, Brikowski et al, 2008, Romero et al, 2010, Bidyalakshmi et al, 2024).

COX is one the main component of renal stones. Renal stones are formed as a result of increased urinary supersaturation, which leads to the formation of crystalline particles. Supersaturation is the determinant for crystallization in fluids like urine. When a solvent is introduced to a salt, the salt dissolves until it reaches a specific concentration, beyond which it is difficult for the salt to dissolve any more. At this stage, the solvent is considered to be salt saturated. As temperature and P^H remain constant, more salt crystallizes in solution. If crystallization blockers are ineffective, nephrosis will develop (Aggarwal et al, 2013, Carvalho et al, 199).

Epidermiological studies showed that diet could be one the main risk in protein can cause a melioration of metabolic acidosis- attenuating further nephropathy progression in patients with chronic kidney disease and reduction of glomerular filtration rate (GFR) (Curhan et al, 2004). It has been found that regular intake of natural diets rich in fruits can increase urine pH and volume, as well as, the amounts of stone inhibitors such as phytate, citrate, potassium and magnesium which are associated with supersaturation of COX and uric acid (Taylor et al, 2004, Meschi et al, 2012). Phytate is the main form of phosphate in natural sources and its dietary intake is associated with the development of insoluble complexes with calcium within the gout, which can cause suppression of crystal formation in the urine and decrease the risk of urolithiasis (Mina et al, 2018, Meschi et al, 2004 and Bhagavathula AS et al). Alkali load induced by a natural diet is able to raise urinary citrate which has a significant preventive effect on the development of kidney stones (Meschi et al, 2004). Additionally, dietary fiber which is abundant in fruits can diminish the formation of stones due to its non-digestible ingredients which link to minerals and fat within the gastrointestinal tract, resulting in the suppression of urinary excretion of oxalate and calcium (Aggarwal et al, 2013). Now, it can be concluded that higher intake of fruits was related to a decreased risk of development of urolithiasis (Kumar et al, 2009).

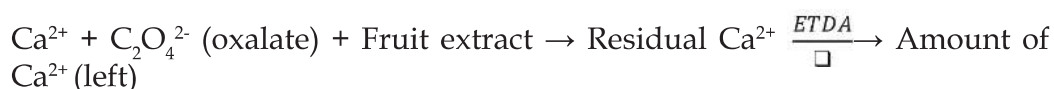
The role of medicinal plants and vegetable diet, fruits have been a growing resurgence as – they are regarded as cost effective, readily available and have reduced side effects. With reference to the latest study reports, higher intake of fruits plays an important role in the prevention of urolithiasis (Mina et al, 2018, Seltzer et al, 1996, Sorensen et al, 2014). This is because of

the reason that fruits are rich in vitamins, antioxidants, antidiabetic property, minerals, antiurolithiatic property, etc. That is why we are trying to focus on medicinal plants mainly fruits for their chemoinhibitory effects on COX stone formation both in aqueous and urinary media. If some fruits are found to be effective in the chemoinhibitory effects (antiurolithiatic property) on COX stone formation, it will be very helpful to the general public.

2. Materials and Methods

2.1. Methodology

The main objective of our research work is to find out which fruits has the highest chemoinhibitory power on the COX stone (kidney stone) formation in the aqueous or urinary medium. It is known that COX stone is formed by mixing calcium ions and oxalate ions. This formation of COX stone may be reduced, blocked and negative effect at all in the presence of fruit extract in the aqueous and urinary media. An experimental model is set up in such a way that two salt forming solution – CaCl_2 and $\text{Na}_2\text{C}_2\text{O}_4$ for COX are allowed to fall simultaneously and slowly drop wise with equal speed into a 250ml beaker containing 50ml of inhibitor solution (fruit extract) in the aqueous or urinary medium. At the ends, the contents 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 is determined by complexometric titration method using standard EDTA solution.



while calculating the calcium content of centrifugate, a titre value of EDTA vs. the corresponding total inhibition solution was deduced from the total titre values (equivalent to centrifugate). It can be concluded that if the $\text{Ca}^{2+}(\text{left})$ is higher, it means that the particular fruit has a higher chemoinhibitory effect. Therefore, chemoinhibitory power of the fruits can be compared and which fruit is the best chemoinhibitory effect. Thus, this particular fruit may be useful for those patients which have kidney stone problems. Therefore, the following relations (1) and (2) are used for the calculation of % inhibition and % increase of Inhibition relative in blank.

Inhibition efficiency (i.e. % inhibition) was calculated by the following equation.

$$\% \text{ Inhibition} = \frac{\text{Ca}^{2+} \text{ in centrifugate}}{\text{Total Ca}^{2+} \text{ in the experiment}} \quad (1)$$

$$\text{Thus, \% increase of Inhibition relative in blank} = \frac{\text{Increase of \% inhibition}}{\% \text{ Inhibition by blank}} \quad (2)$$

where the total Ca^{2+} in the experiment equals the Ca^{2+} contents of 50ml CaCl_2 solution which is determined separately.

Manipur is rich in Flora and fauna. Different types of local fruits are found in different districts (16 districts) of Manipur. Therefore, healthy fruits are procured from the IMA markets and different districts. Fruits were washed, dried, chopped and powdered. The dried powder parts of the fruits were soaked in 50% aqueous methanol (1:1) in a soxhlet extractor under hot condition. The fruit extracts were distilled under reduced pressure using Rotary Vacuum Evaporator(RII) to produce crude mass which further spread in Petridis and dried in the desiccators. Table1 gives the scientific names, local names and the part of the fruits used.

Table 1: Fruits with their particulars

Sl. No.	Scientific name	Local name	Parts of fruit used
1	<i>Averrhoa carambola</i>	Heinajom (Figure 1)	Pulp
2	<i>Garcinia pendunculata</i>	Heibung (Figure 2)	Pulp
3	<i>Citrus latipes</i>	Heiribob (Figure 3)	Pulp
4	<i>Embllica officinaus</i>	Heikru (Figure 4)	Pulp
5	<i>Anannas comosus</i>	Pine apple	Pulp
6	<i>Solanum nigrum</i>	Leipung khanga	Pulp
7	<i>Aegle marmelos</i>	Heirikhagok	Pulp
8	<i>Ficus glomerata</i> Roxb	Heibong	Pulp
10	<i>Celtis australis</i>	Heikreng	Pulp



Figure 1: *Averrhoa carambola*
(Heinajom)



Figure 2: *Garcinia pendunculata*
(Heibung)

Figure 3: *Citrus latipes* (Heiribob)Figure 4: *Emblica officinaus* (Heikru)

2.3. Collection of Urine

Chemoinhibitory experiment is carried in the aqueous as well as in the urinary medium. Thus urine was collected from a healthy man (~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 and fresh urine was always used for our research purposes.

2.4. Chemoinhibitory Experiment

Chemoinhibitory experiments of the fruit extracts, including blank reading both in aqueous and urinary media, were conducted according to Rao TVRK (Rao TVRK et al, 2008). 0.01M each of CaCl_2 and sodium oxalate were taken for COX crystallization. 50ml of fruit extract (0.1%) in water or urine was taken as inhibitor solutions. Simultaneous blank experiments water or urine were also carried out for calculating the inhibitory efficiency of fruit extract compared to water or urine (Table 2). All the experiments were conducted at room temperature (25°C). At the end of crystallization, the content of the beaker was digested on a hot water bath for 10 minutes, cooled at room temperature and centrifuged in small volume. The total centrifugates were collected. The 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 $\text{NH}_3\text{-NH}_4\text{Cl}$ as buffer (pH10). While calculating the Ca content of the centrifugate, a titre value of EDTA versus inhibition corresponding total inhibition solution was deduced from the total titre value.

Table 2: Inhibition experiment for COX formation in blank solution – water and urine

Sl. No.	Water – Blank for COX				Urine – Blank for COX			
	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	2.5	
2	0	1.2	1.2	1.2	0	2.0	2.5	2.5
3	0	1.2	1.2		0	2.0	2.5	

Table 3: Effect of fruit extract on COX formation in aqueous medium

Plant (fruit)	Inhibitors 0.1%	Ca ²⁺ in solution (g)	Ca ²⁺ in precipitate (g)	% of Inhibition	Diff % of Inhibition between sample and blank	Relative % of Inhibition
Blank	H ₂ O BR =1.2	0.0008 ×1.2 =0.0010	0.0735 -0.0010 =0.0725	0.0010×100÷0.0735 =1.3605	-	-
<i>Averrhoa carambola</i>	Crude BR =1.66	0.0008 ×1.66 =0.0013	0.0735 -0.0013 =0.0722	0.0013×100÷0.0735 =1.7687	1.7687 -1.3821 =0.3866	0.3866×100 ÷1.3821 =27.9719
<i>Garcinia pendunculata</i>	Crude BR =1.7	0.0008 ×1.7 =0.0014	0.0735 -0.0014 =0.0721	0.0014×100÷0.0735 =1.9048	1.9048-1.3605 =0.5443	0.5443×100 ÷1.3605 =40.0074
<i>Citrus latipes</i>	Crude BR =1.9	0.0008 ×1.9 =0.0015	0.0735 -0.0015 =0.0720	0.0015×100÷0.0735 =2.0408	2.0408 -1.3605 =0.6803	0.6803×100 ÷1.3605 =50.0037
<i>Embllica officinaus</i>	Crude BR =2.3	0.0008 ×2.3 =0.0018	0.0735 -0.0010 =0.0717	0.0018×100÷0.0735 =2.4490	2.4490 -1.3605 =1.0885	1.0885×100 ÷1.3605 =80.0074
<i>Anannas comosus</i>	Crude BR =1.7	0.0008 ×1.7 =0.0014	0.0735 -0.0014 =0.0721	0.0014×100÷0.0735 =1.9048	1.9048 -1.3605 =0.5443	0.5443×100 ÷1.3605 =40.0074
<i>Solanum nigrum</i>	Crude BR =1.5	0.0008 ×1.5 =0.0012	0.0735 -0.0012 =	0.001×100 ÷0.0735 =1.6323	1.6323 -1.3605 =0.2718	0.2718×100 ÷1.3605 =19.9779
<i>Aegle marmelos</i>	Crude BR = 3.6	0.0008 ×3.6 =0.0029	0.0735 -0.0029 =0.0706	0.0029×100 ÷0.0735 =3.9456	3.9456 -1.3605 =2.5851	2.5851×100 ÷1.3605 =190.0110
<i>Ficus glomerata Roxb</i>	Crude BR = 3.6	0.0008 ×3.6 =0.0029	0.0735 -0.0029 =0.0706	0.0029×100÷0.0735 =3.9456	3.9456 -1.3605 =2.5851	2.5851×100 ÷1.3605 =190.0110
<i>Celtis australis</i>	Crude BR = 4.5	0.0008 ×4.5 =0.0036	0.0735 -0.0036 =0.0699	0.0036×100÷0.0735 =4.8980	4.8980 -1.3605 =3.5375	3.5375×100 ÷1.3605 =260.1470
Cystone	1.5	0.0008 ×1.5 =0.0012	0.0735 -0.0012 =0.0723	0.001×100 ÷0.0735 =1.6323	1.6323 -1.3605 =0.2718	0.2718×100 ÷1.3605 =19.9779

Table 4: Effect of fruit extract on COX formation in urinary medium

Sl. No.	Plant (fruit)	Inhibitors 0.1%	Ca ²⁺ in solution (g)	Ca ²⁺ in precipitate (g)	% of Inhibition	Diff % of Inhibition between sample and blank	Relative % of Inhibition
1	Blank	Urine BR = 2.5	0.0008 ×2.5 =0.0020	0.0735 -0.0020 =0.0715	0.0020×100 ÷0.0735 =2.7211	-	-
2	<i>Averrhoa carambola</i>	Crude BR = 4.7	0.0008 ×4.7 =0.0038	0.0735 -0.0038 =0.0697	0.0038 × 100 ÷ 0.0735 =5.1701	5.1701 -3.4014 =1.7687	1.7687×100 ÷5.1701 =34.2111

3	<i>Garcinia pendunculata</i>	Crude BR = 4.8	0.0008 × 4.8 =0.0038	0.0735 -0.0038 =0.0697	0.0038 × 100 ÷ 0.0735 =5.1701	5.1701 -2.7211 =2.4494	2.4494×100 ÷2.7211 =90.0151
4	<i>Citrus latipes</i>	Crude BR = 7.03	0.0008 × 7.03 =0.0058	0.0735 -0.0058 =0.0667	0.0058 × 100 ÷ 0.0735 =7.8912	7.8912 -2.7211 =5.1701	5.1701×100 ÷2.7211 =190.0004
5	<i>Embllica officinaus</i>	Crude BR = 3.0	0.0008 × 3.0 =0.0024	0.0735 -0.0024 =0.0711	0.0024×100 ÷0.0735 =3.2653	3.2653 -2.7211 =0.5442	0.5442×100 ÷2.7211 =19.9993
6	<i>Anannas comosus</i>	Crude BR = 2.7	0.0008 × 1.7 =0.0022	0.0735 -0.0022 =0.0713	0.0022×100 ÷0.0735 =2.9932	2.9932 -2.7211 =0.2721	0.2721×100 ÷2.7211 =9.9996
7	<i>Solanum nigrum</i>	Crude BR = 3.4	0.0008 × 3.4 =0.0027	0.0735 -0.0027 =0.0708	0.0027×100 ÷0.0735 =3.6735	3.6735 -2.7211 =0.9524	0.9524×100 ÷2.7211 =35.0006
8	<i>Aegle marmelos</i>	Crude BR = 5.05	0.0008 × 5.05 =0.0040	0.0735 -0.0040 =0.0695	0.0040×100 ÷0.0735 =5.4422	5.4422 -2.7211 =2.7211	2.7211×100 ÷2.7211 =100.0000
9	<i>Ficus glomerata Roxb</i>	Crude BR = 3.5	0.0008 × 3.5 =0.0028	0.0735 -0.0028 =0.0707	0.0028×100 ÷0.0735 =3.8095	3.8095 -2.7211 =1.0884	1.0884×100 ÷2.7211 =39.9985
10	<i>Celtis australis</i>	Crude BR = 7.1	0.0008 × 7.1 =0.0057	0.0735 -0.0057 =0.0678	0.0057×100 ÷0.0735 =7.7551	7.7551 -2.7211 =5.0340	5.0340×100 ÷2.7211 =184.9987
11	Cystone	BR = 4.1	0.0008 × 4.1 =0.0033	0.0735 -0.0033 =0.0702	0.0033 ×1000.0735 =4.4898	4.4898 -2.7211 =1.7687	1.7687×100 ÷2.7211 =64.9994

3. Results and Discussion

We have performed the chemoinhibition experiment in both aqueous and urinary media to calculate the percentage of inhibition (Table 2). Using the titre value 1.2(aq) and 2.5(Urinary), the Ca^{2+} in solution, Ca^{2+} in COX precipitate were calculated which were used in the calculation of relative percentage of inhibition for the experimental fruit extract (0.1% strength) in table 3 and table 4 respectively. The effect of inhibition in COX commercially available fruits by Herbal Drug Cystone, manufactured by Himalaya Drug Company was also tested and compared with experimental fruits extract in both medium (Table 2 and Table 3). Table 2 and 3 show the calculation for how much Ca^{2+} ion was utilized in the formation COX ppt. Larger is the value of Ca^{2+} in the formation of COX ppt, weaker is the chemoinhibitory effect. Table 5 gave an evaluation on the comparative values of chemoinhibitory effects of COX stone. Table 5 shows the comparison of the antiurolithiatic(Basavaraj DR et al, 2007) properties of the fruits with Cystone. Some fruits are found to have higher antiurolithiatic property in the aqueous and urinary media while some fruits have lower antiurolithiatic property. But some fruits show negative results. Further as compared to the antiurolithiatic property of Cystone, some fruits show higher antiurolithiatic property while some fruits show lesser antiurolithiatic property.

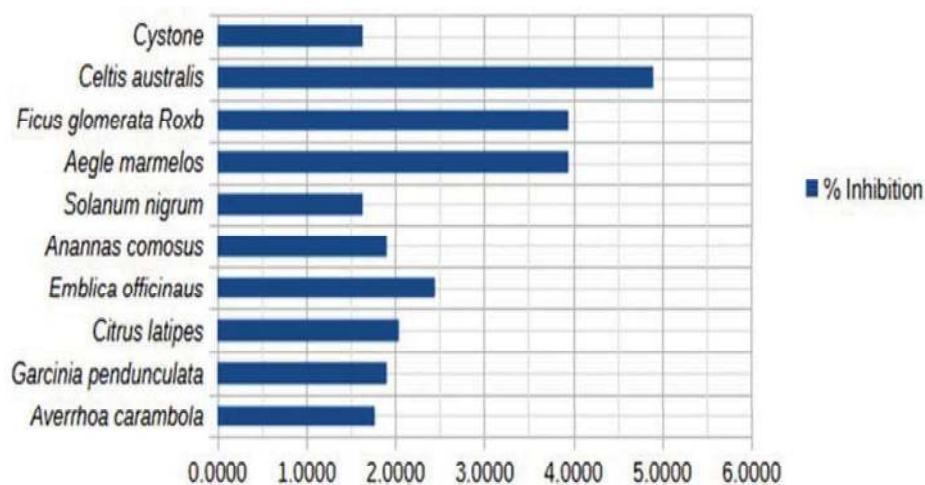


Chart 1: % inhibition for different fruit extracts (0.1% strength) in aqueous medium

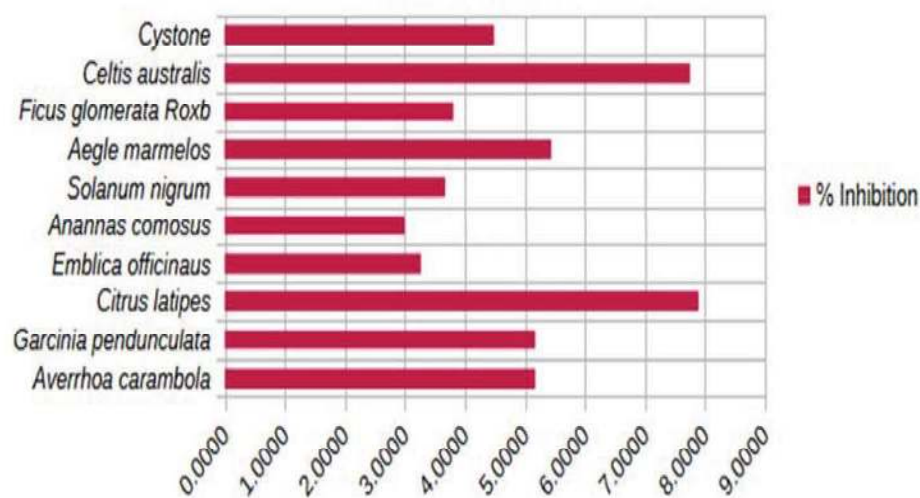


Chart 2: % inhibition for the different fruit extracts (0.1% strength in urinary medium)

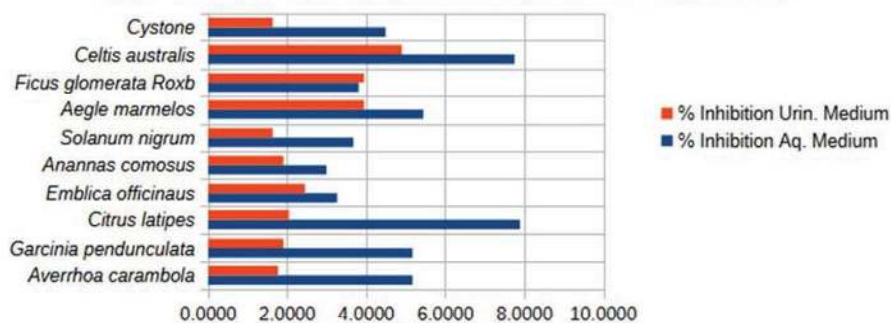


Chart 3: Comparison of % inhibition for the different fruit extracts (0.1% strength) between aqueous and urinary media

Table 5: Comparison of chemoinhibitory effects on COX stone formation

Sl. No.	Name of drug/ plant(fruit)	Type of stone	Aqueous medium		Urinary medium	
			% Inhibition	% Relative inhibition	% Inhibition	% Relative inhibition
1	Cystone	COX	1.6323	19.9779	4.4898	64.9994
2	<i>Averrhoa carambola</i>	COX	1.7687	27.9719	5.1701	51.9992
3	<i>Garcinia pendunculata</i>	COX	1.9048	40.0074	5.1701	51.9992
4	<i>Citrus latipes</i>	COX	2.0408	50.0037	7.8912	190.0004
5	<i>Embllica officinaus</i>	COX	2.4490	80.0074	3.2653	19.9993
6	<i>Anannas comosus</i>	COX	1.9048	40.0074	2.9932	9.9996
7	<i>Solanum nigrum</i>	COX	1.6323	19.9779	3.6735	35.0006
8	<i>Aegle marmelos</i>	COX	3.9456	190.0110	5.4422	100.0000
9	<i>Ficus glomerata</i> Roxb	COX	3.9456	190.0110	3.8095	39.9985
10	<i>Celtis australis</i>	COX	3.5375	260.1470	5.0340	184.9987

Findings from the chemoinhibitory inhibitory experiments show that the antiurolithiatic property of the fruits were more than blank aqueous and blank urine. Therefore, it is noticed that the fruit extracts have greater antiurolithiatic property for COX stone formation in the urinary medium. Further, it is shown that the inhibitory effects eg. *Garcinia pendunculata* (5.1701 in urinary medium) in the mineralization of stone forming chemicals in urine were more than that in aqueous medium (1.7687 in aqueous medium of the same fruit) except some fruits. Hence, it is learned that there may be some natural inhibitors in the urinary medium. Among the fruits studied, *C.australis* has the highest antiurolithiatic property on COX stone formation in aqueous medium while *C.latipes* has the antiurolithiatic property on COX stone formation in the urinary medium. From the overall data, *C. australis* and *C.latipes* have the highest antiurolithiatic property on COX stone formation.

Further, as compared to antiurolithiatic property of Cystone, a herbal drug, with that of has lower antiurolithiatic property than that of fruits in the aqueous medium. In the urinary medium, Cystone has higher antiurolithiatic property than some fruits (Table 3) but some fruits have higher antiurolithiatic property (2 or 3 times) than Cystone (Table 4). Some fruits are rich in phytochemical compounds like coumarins, flavonoids, mucilages, terpenoids, siteroids, etc. are present (Ramesh et al, 2022, Ranjan et al, 2011, Goa et al, 2015, Xie et al, Chen et al, 2017) Fruits are also rich in minerals like Ca, K, Zn, Mg, etc. (Ranjan et al, 2011). Since these fruits have the antiurolithiatic properties, the phytochemical compounds present in these fruits may have certain roles in the antiurolithiatic property. Further, among the minerals, Ca plays important role in the antiurolithiatic property. Hence, chemical elements (minerals) and compounds present in these fruits play significant role in the biological activities directly or indirectly. The mechanism of chemoinhibitory effect (antiurolithiatic property) of

these fruits is yet to find out whether either minerals or phytochemical compounds present in the fruits or both act as antiurolithiatic activities. Minerals or compounds enhance the digestion or breaking down of the COX stone (kidney stone) or inhibit the formation of kidney stone. According to Bimola(Bimola et al, 2016)Ca(2960mg/100g in *C.Javana* DC) enhances the antiurolithiatic property on COX stone formation and for this reason it can be presumed that Ca is also a competitive inhibitor of oxalate in COX stone formation(Bimola DA et al, 2016). Further, high Ca content lowers the amount of oxalate absorbed into blood thereby reducing risk of new kidney stone. And in this context, *Celtis australis* being containing high Ca may have the higher antiurolithiatic property on COX stone (kidney stone) in the urinary medium. Thus, this is due to the fact that phytochemical compounds help in the antiurolithiatic property on COX stone. The reason is that these chemical compounds may help either to remove Ca from COX or breaking down of oxalate from COX. Further investigation is required because which chemical compounds are actually involved in the blocking of COX stone formation. Therefore, the chemoinhibitory activity on COX stone formation (kidney stone) in the human body by using fruits/medicinal plants was done by our traditional healers (folklore medicine) and still this practice is going on by local herbalists like Shri Laishram Nabakishore Singh, a Padma shree awardee(awarded Padma Shree on 2001)(Oinam et al, 2023).

Conclusion

It is noticed from the experimental data of the ten fruits studied that almost all the fruits are ethanomedicinally important because of their positive chemoinhibitory(Antiurolithiatic) properties. These fruits contain bioactive phytochemical compounds, Vitamin C, rich minerals like Ca, K, Mg, Fe, etc. If this research work is studied together with the pharmaceutical experts, the outcomes will be very fruitful and useful to the general public. Further our future scope is the isolation of the bioactive compounds including minerals responsible for the antiurolithiatic effect and identification of specific compounds for pharmacological studies. We will try to find out the actual mechanism of the dissolution or prevention of the formation of kidney stones i.e. calcium oxalate stone. This will help in the designing of drug and validation of the antiurolithiatic effect in controlled biological system like rat model and lastly to animal model like human being.

Statistical Interpretation

In this investigation, we utilized correlation statistics along with their associated P-values to examine the relationships between Inhibition (0.1%), the concentration of Ca^{2+} in solution (g), and the concentration of Ca^{2+} in precipitate (g) across twelve study subjects like Blank-Water, Blank-Urine, *Averrhoa carambola*, *Garcinia pendunculata*, *Citrus latipes*, *Emblica officinaus*,

Anannas comosus, *Solanum nigrum*, *Aegle marmelos*, *Ficus corus*, *Celtis australis* and Cystone(Standard). When investigating the effect of fruit extract in aqueous and urinary media, a highly significant linear relationship was observed between Inhibition (0.1%) and the concentration of Ca^{2+} in solution (g). However, weak linear relations ($P>0.05$) were observed between Inhibition (0.1%) and Ca^{2+} in precipitate (g), as well as between the concentration of Ca^{2+} in solution (g) and Ca^{2+} in precipitate (g).

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Dawson CH, Tomson CR, Kidney Stone disease, pathology, investigation and medical treatment. Clin Med(Lond). 2012;12(5):467-71, doi: 10.7861/Clinmedicine.12-5-167.
2. Moe OW, Kidney stones: pathphysiology and medicinal management. Lancet. 2006;367(9507):333-344. doi: 10.1016/S0140-6736(06)68071-9
3. Younis A, Radhakrishnan S. Ytends Urol Men's Health. 2011; 2:15-20. doi: 10.1002/tre.197.
4. Han H, Segal Am, Seifler JL, Dwyer JT, Nutritional management of kidney stones(Nephrolithiasis),Clin Nutr Res. 2015;4(3):137-152 doi:10.7762/enr.2015.4.3.137.
5. Mahmoud NAMRKNKSA, Bahmani. Medicinal plants for treatment of kidney stones, An ethnobotany study in Shahrekord. Egyptian J Vet Sci. 2019;50(2): 145-149. doi:10.21608
6. Alelign T, Petros B, Kidney stone disease: Anupdate on current concepts. Adv Urol. 2018,P.3068365-3068365. doi: 10.1155/2018/3068365.
7. Coe Fl, Evan A, Worcester E. Kidney stone disease. J Clin Invest. 2005;115(10): 2598-2608. doi: 10.1172/Jcl26662.
8. Bihl G, Meyers A. Recurrent renal stone disease advances in pathogenesis and clinical management. Lancet. 2001;358(9282):651-656. doi: 10.1016/S0140-6736(01)05782-8.

9. Bhutani KK, Goyal A & Singh S, Herbal Wealth of Northeast India: A pictorial and herbaria guide. Department of Natural Products, National Institute of Pharmaceutical Educational and Research 2008, S.A.S. Nagar, Punjab, Ludhiana. Doi:10.1038.
10. Yadav RD, Jain SK, Alok S, Mahor A, Barb JP, Jaiswal M. Herbal plants used in the treatment of urolithiasis: a review. *Int J Pharm Sci.* 2011;2(6):1412-1420. Doi: 10.47583
11. Kasole DM, Jagtap SD, Thapa D, Khyade MS, Russell WR. Herbal remedies for urinary stones used in India and China: A review *J Ethnopharmacol.* 2017;203:55-68. doi:10.1016/j.jep.2017.03.038.
12. Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest.* 2005;115(10): 2598-2608. doi: 10.1172/Jci26662.
13. Bihl G, Meyers A. Recurrent renal stone disease advances in pathogenesis and clinical management. *Lancet.* 2001;358(9282):651-656. doi: 10.1016/S0140-6736(01)05782-8.
14. Kasole DM, Jagtap SD, Thapa D, Khyade MS, Russell WR. Herbal remedies for urinary stones used in India and China: A review *J Ethnopharmacol.* 2017;203:55-68. doi:10.1016/j.jep.2017.03.038.
15. Anamika U, Chaturvedi SS, Tiwari BK, Department of Environmental Studies, NEHU, Shillong -793022, Meghalaya, Utilization of wild Citrus by Khasi and Garo tribes of Meghalaya, Mohammed I, Rasool, Tariq H. Mousa Z, Hayjaa M. *Jan*2016; 15(1):121- 7, doi: 10.56042.
16. Muharrem, Lutfi & Ugur C, An ethnobotanical survey of medicinal plants in Karliova(Bingo-Turkey), *Ind.J.Tradit.Know.* 2019 ;18(1):76-87, doi:10.56042.
17. Sheikh Z, Raees K, Alushtaq A, Muhammad ZB, Muhammad Z. Ethnobotanical survey of highly effective medicinal plants and phytotherapies to treat diabetes mellitus II in South-west Pakistan, *Ind.J.Tradit.Know.*2018 ; 17(4): 682-690, doi:10.56042.
18. Mohammed I, Rasool, Tariq H. Mousa Z, Hayjaa M. Potential of medicinal plants for the management of renal stones, *Bagh. J. of Biochem. and Appl. Bio. Sci.*2022; 3(2):69-98(e-ISSN,2706-9915),doi:10.47149.
19. Ankur C, Amarchand P, Aadarsh C, Deepa I, S PR, K PV. Potential of medicinal plants in kidney, gall and urinary stone. *Int J Drug Dev Res.* 2010;2(2): 431-447. doi: 10.36648.
20. Basavaraj DR, Biyani CS, Browning Aj, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. *EAU-EBU Update Ser.* 2007;5(3)126-136. doi: 10.1016/j-eeus.2007.03.002.
21. Stamatelou KK, Francis ME, Jones CA, Nyberg LM. Curhan GC. Time trends in reported prevalence of kidney stones in the United States. 1976-1994. *Kidney Int.* 2003;63(5): 1817-1823. doi: 10.1046/j.1523-1755.2003.00977.
22. Shu X, Cai H, Xiang YB,. Green tea intake and risk of incident kidney stone: Propective cohort studies in middle aged and elderly chinese individuals. *Int J Urol.* 2019;26(2):241-246. doi: 10.1111/iju.13849.

23. Mahmoud N & Bahmani, Medicinal Plants for treatment kidney stones, An ethnobotany study in Shahrekord, Egy.J. vet. Sci.2009;50(2):145-152,doi:10.21608.
24. Brikowski Th, Lotan Y, Pearls MS, Climate- related increase in the prevalence of Urolithiasis in the United States. Proc.Natl.cad. Sci. USA.2008;105(28):9841-6, doi:10.1073.
25. Romero V, Akpiner H, Assimos DG, Kidney stones: prevalence, incidence and associated risk factors, Rev.Urol. 2010; 12(2):e86-e96,doi:10.3909.
26. Bidyalakshmi MD & Ashalata D, Estimation of phytochemical constituents and antioxidant potential of underutilized wild edible fruits of Manipur, Mohammed I, Rasool, Tariq H. Mousa Z, Hayjaa M.May2024 ;33(5):478-488, doi: 10.56042/ijtkv23i5.11041.
27. Aggarwal KP, Narula S, Kakkar M & Tandon C. Nephrosis: molecular mechanism of renal stone formation and the critical role played by modulators. Biomed.Res. Int.2013;292953-55. Available from, doi:10.1155/2023/292953
28. Carvalho M, Nakagawa Y. Urinary supersaturation and recurrence in nephrolithiasis. Int. Braz. Urol.1999; 25:475-9, doi:10.1590.
29. Curhan GC, Willett WC, Knight EL, Stampfor MJ. Dietary factors and the risk of incident kidney stones in younger women: Nurses' Health Study II, Arch. Inter. Med. 2004;164:885-91, doi:10.1001/archinte.164.8.885[PubMed][CrossRef][Google Scholar].
30. Taylor EN, Stampfor MJ, Curhan GC, Dietary factors and the risk of incident kidney stones in men: New insight after 14 years of follow up. J.Am.Soc. Nephrol.2004;15:3225-32, doi: 10.1681
31. Meschi T., Nouvenne A, Ticinesi A, Prati B, Guerra A .Dietary habits in women with recurrent idiopathic calcium nephrolithiasis, J.Transl.Med.2012;10:63. doi:10.1186/1479-5876-10-63[PMC free article][PubMed][CrossRef][Google Scholar].
32. Mina CN, Marziyeh IH, Roja R, Mohammad HSZ, Seyed MN & Anupam B. Dietary plants for the prevention and management of kidney stones: Preclinical evidence and molecular mechanisms, Int. J. Mo. Sci. Mar2018 19(3):765.doi:10.3390.
33. Meschi T, Maggiore U. Fiaccadori E., Schianchi T., Bosi S. The effect of fruits and vegetables on urinary stone risk factors. Kidney Int. 2004; 66:2402-10. doi:10.1111/j.1523-1755.2-04.66029.x.[PubMed][CrossRef][Google Scholar].
34. Bhagavathula AS, Al-Khatib M, Elnour Aj, AA, Kalbani A, Shehab NM, et al. Ammi visnaga in treatment of urolithiasis and hypertriglyceridemia. Pharmconosy Res.2014;7(4):397-400. doi:10.4103/0974-8490-167894.
35. Kumar V, Sinha AK, Makhar HP & Becker K. Dietary roles of phytate and phytase in human nutrition: A review. Food Chem.2010;120:94559.doi:10.1016/j.foodchem.2009.11.052[CrossRef][Google Scholar]
36. Seltzer MA, Low RK, McDonald M, Shami GS & Stoller ML. Dietary manipulation with lemonade to treat hypocitraturic calcium nephrolithiasis, J. Urol. 1996;156:907-9.doi:10.1097.

37. Sorensen MD, His RS, Health initiative report. J. Urol. 2014;192:1694-99. doi:10.1016/j.juro.2014.05.086[PMC free article][PubMed][CrossRef][Google Scholar]
38. Rao TVRK, Choudhury VK. Chemoinhibition of Minerealization of Urinary stone formation by some inorganic and organic salts of Aluminium in Aqueous and Urinary Media, Asian J. of Chem. 2008 ;20(7):5046 -52. doi: 1014233.
39. Basavaraj DR, Biyani CS, Browning AJ, Cartledge JJ. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. EAU-EBU Update Ser. 2007;5(3):126-136. doi: 10.1016/j.eeus.2007.03.002.
40. Ramesh KS, Arina R, Kavita S, Parchuri P, Xiaomin S. Bioactive compounds of citrus fruits: A review of composition and health benefits of carotenoids, flavonoids, limonoids and terpenes. Antioxidant(Basel), Feb2022;11(2):239, doi:10.3390/antiox11020239.
41. Ranjan S, Gokila M, Brinda P & Sujatha RK. Antioxidant and Phytochemical properties of *Aegle marmelos* fruit pulp, Int. J. of Current Pharm. Res. 2011;3:65-70, doi:10.22159.
42. Goa Y, Huany R, Gong Y, Park HS, Wen Q. The Antidiabetic Compounds, 2-dodecyl-6-methoxycyclohexa-2,5-diene- 1,4-dione, Isolated from *Averrhoa carambola* L., Demonstrates significant Antitumor potential against Human Breast Cancer Cells. Oncotarget. 2015; 6(27):24304-24319, doi:10.18632/oncotarget.4475.
43. Xie, Q, Zhang, S, Chen, C, Li, J, Wei, X, Xu, X. Protective Effect of 2-dodecyl-6-methoxycyclohexa-2,5-diene- 1,4-dione, Isolated from *Averrhoa carambola* L., Against Palmitic acid-Induced inflammation and Apoptosis in Min6 Cells by inhibiting the TLR4 - MyD88-NF-KB Signalling Pathway, Cell Physiol. Biochem. 2016;39(5):1705-15, doi:10.1159/000447871.
44. Chen CX, Nong ZH, Xie QQ, He JH, Cai WE, Tang. 2-dodecyl-6-methoxycyclohexa-2,5-diene- 1,4-dione inhibits the Growth and Metastasis of Breast Carcinoma in Mice. Sci. Rep. 2017;7(1):6704. doi:10.1038/41598-017-07162-3.
45. Bimola DA, Warjeet SL, Ibopishak SO, Jeena Th. The isolation of compounds from the aqueous methanol extract of *Cissus javana* DC leaves and determination of its trace element content through wet digestion. Asian J. of Chem. 2016; 26(13):3820-3822, doi: 10.14233.
46. Oinam IS, Asem BD, Rajkumar HS. Evaluation of the effects of *Orthosiphon spiralis*(Lour) Merr., *Cissus Javana* DC., and *Upupa epops* L., on kidney stone along with cysteine – a herbal drug. J. Trad. Folk Pract. 2023;10(01&02):10-7, doi:10.25173.

Author Contribution

Nehru S Khundrakpam and Sonia Th: Literature survey, Field visit, Experimental observation and analysis and Writing – the original paper(draft); Leichil O: Purchasing and collection of fruits and Experimental work as a part of Inspire project, Bimola D Asem: Overall inspection and supervised the experimental data, Methodology, and Writing – reviewing and Ibopishak S Oinam: finalized the paper.