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In-Vitro Study on Anti-Urolithiatic of Brassica Oleracea Var Capitata Linn

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ABSTRACT

Background: Urolithiasis is a condition that is caused by the formation of urinary calculi in the renal tubule, it can be defined as the formation of crystals in the urinary tract. Around 4- 15% of the world population suffer from urinary stones. Urolithiasis is caused by many different factors such as heredity, diet, metabolic abnormality, infection, and slightly by age, gender and climate.

Method: The methodologies used to perform this study *in-vitro* anti-urolithiatic activity analysis by performing microscopic growth of calcium oxalate stone, nucleation assay and aggregation assay on Brassica oleracea var. capitata Linn.

Result: The inhibition of crystal growth by the plant extract is in a dose dependent manner ($P< 0.05$).

Conclusion: The anti-urolithiatic activity of Brassica oleracea var. capitata Linn was experimentally proven.

Keywords: Urolithiatic, Brassica oleracea var. capitata Linn, Nucleation assay, Aggregation assay.

INTRODUCTION

Urolithiasis is a condition that is caused by the formation of urinary calculi in the renal tubule, it can be defined as the formation of crystals in the urinary tract. The stones vary in shape, size, and characteristics of crystal formation. It is formed by the Precipitation of minerals and urinary constituents such as calcium oxalate, uric acid, cysteine, calcium phosphate, and struvite. Around 4- 15% of the world population suffer from urinary stones [1]. The stones most of the time affect the pelvis, calyces, ureter, bladder, and urethra. They are more frequently occurring in men than women 13% and 7 % respectively. [1].

The word “Urolithiasis” is derived from Greek as “Urone” for urine and “Lithos” for stones. Urolithiasis is one of the major diseases of the urinary tract with increasing prevalence and incidence in the world. This urologic disorder occurs in approximately 12% of the global population and it is more common in male than female [2]. Its recurrence rate is high 14% after one year, 25%-31.5% after five years, 49%-52% after ten years, 72% after twenty years [3].

Urolithiasis is caused by many different factors that are related to the development of stone in the urinary tract such as heredity, diet, metabolic abnormality, infection, and slightly by age, gender and climate. Heredity is related to Cysteine stones which are caused by a rare disorder called cystinuria inherited from one parent to a child [4]. Cystinuria, an inherited or genetic disorder where transporting amino acid (cysteine) which are building blocks of protein which result in the excess amount of cystine in the urine (cystinuria) and the formation of cystine stones. [5] They are uncommon when compared to the other kinds of stones, they consist of only 1%-2% of adult urinary tract disease and 10% of children's disease [4]. Even though cystine is not the only amino acid excreted in cystinuria it is difficult to solubilize than all of the naturally soluble amino acids. Its precipitation out of urine tends to form urinary calculi in the urinary tract. The smaller stones tend to pass in urine while the bigger stones are difficult to pass and cause nephrolithiasis.

The exact flow of the pathology of urolithiasis is still unknown.it mainly depends on the stone phenotype in the tract. In urolithiasis caused by CaOx, the decrease in solubility with increasing uric acid levels result in the nucleation of CaOx. When precipitation and buildup of CaOx in the sub epithelial space and basement membrane of renal papillae causing Randall plaques to result in the generation of calculi.

Consumption of Diet or foods which are rich in oxalates such as nuts, beets, spinach, seeds, and buckwheat seems to have the greater possibility of causing calcium oxalate stone. A metabolic abnormality that may lead to urolithiasis includes hypercalciuria, hypokalemia, hyperuricemia, hyperuricosuria, hypophosphatemia, and low urine volume [6]. The cause of stone disease varies in different population and environmental and

genetic factors might result in these differences
Stress-induced secretion of vasopressin and Adrenocorticotropic hormone (ACTH) results in hypertonic urine and elevated serum calcium levels which explain the mechanism of stress as a pathophysiological factor in inducing the CaOx urolithiasis.

In struvite stone pathogenesis, increasing pH due to microbial conversion of urea to carbon dioxide and ammonia is the reason for the crystallization of struvite stones. The other non-urease microbes like E. coli debated that the increased matrix production favors the adherence of struvite crystals at the site of calculi induction. In uric acid stones, the pathological mechanisms seem to be various in nature. It may be idiopathic, acquired, and congenital mechanisms in bringing the pathogenesis. Clinically it was apparent that the hyperuricosuria in uric acid stones pathogenesis resulted from the high intake purine-rich diet and drugs that harm the absorption of reabsorption of uric acid. Moreover, hyperuricosuria decreasing the pH is considered idiopathic uric acid nephrolithiasis. In cystine stones, mostly the autosomal recessive genetical abnormality of proximal tubule impairs the reabsorption of cystine by APRT deficiency. Thus the cystinuria becomes a predisposing factor for cystine nephrolithiasis. [7]

Inspecting the medical history of the patient and their family is the first step taken in the diagnosis as that might help in revealing any medical condition that they are suffering from and if they are on any medication. A medical history of renal calculi the family and any nutritional behaviors help in determining the potential cause of the disease. Ideally, pain in the flank and groin, and hematuria may give an idea to the practitioner the presence of stone but to confirm if it is for sure kidney.[8]

Nowadays urolithiasis can be diagnosed and treated in different methods.it can be treated pharmacologically and non-pharmacologically. The pharmacological treatment of urolithiasis in another name is called medical expulsive therapy include the use of drugs such as calcium channel blocker, alpha-blocker and corticosteroid, and most recently phosphodiesterase-5 inhibitors (PDE5) inhibitors. Both calcium channel blockers and alpha-blockers have been used in the expulsion of stone for a long time now. [9]

Alpha-blockers are the most commonly recommended drugs for the expulsion of distal ureteral stones because of lesser pain episodes while passing. Alpha-blockers are muscle relaxants and the wall of the bladder is composed of smooth muscle fiber oriented in multiple different directions, detrusor muscle. The most commonly used alpha-blocker drug is tamsulosin, terazosin, and doxazosin.

Among several kinds of calcium channel blockers the only drug that has been proved to show effective renal colic reducing properties is Nifedipine. The alpha-blockers are more significant than calcium channel blockers in reducing pain while passing the stones, this has made it clear for physicians not to use Nifedipine as monotherapy for medical expulsive therapy except when they are administered along with alpha-blocker.

Corticosteroids are another pharmacological treatment used for the expulsion of stones. Although they are mostly used in combination with drugs that facilitate stone passage in which they have been used to treat or prevent the induced mucosal inflammation which led to the formation of edema in the presence of stone.

For the treatment of large stones can be done by using extracorporeal shock wave lithotripsy (ESWL) which break the large stones into tiny pieces. This therapy is high expensive and may damage to the urinary system [10]. Also, they do not prevent the formation of new stones [11].

Using medicinal plants for the treatment of the urolithiasis is simple, have less side effects and cost-effective. According to the World Health Organization (WHO) about 70% of global populations are using indigenous medicines to cure various diseases.

Plenty of medicinal plants have been using as traditional health care system from the centuries in folk and Ayurvedic treatments. Some medicinal plants have been reported to be used in the treatments of urolithiasis in folk and Ayurvedic medical practices and they have shown a substantial effect on *in-vitro* and *in-vivo* anti-urolithiatic activity in researches. So, this study was carried out for evaluation of *in-vitro* anti-urolithiatic of Brassica Oleracea var capitata Linn. Even though there were differences between naturally occurring and experimentally prepared kidney stones have existed, the study was carried out as an experimental study or as the first step for the drug discovery.

MATERIALS AND METHODS

Plant authentication

The selected plant Brassica oleracea var. capitata Linn was collected from a local market in Vaddeswaram and authenticated by P.Satyanarayana Raju, dept. of Botany and microbiology, Charya Nagarjuna Univers. Nagarajuna Nagar.

Processing of plant material:

The collected plant leaves were washed and dried under the shade of sun and were subjected to grinding to make a coarse powder and fine powder that pass through 100 sieve number and it was then packed and stored at room temperature for further use.

Determination of extractive values:

Preparation of methanol extracts:

The freshly collected selected plant was shredded and dried and made grounded to get a coarse powder. Three hundred grams (300 g) of the powder and soak it into 1200 ml of methanol at the ratio of 1:4 (Powder: Solvent). The mixture was put in storage at room temperature for three consecutive days and the solvent was changed each day by filtering out the previous and store it in a different container after the three-day process the filtrate that has been stored was subjected to heat on water bath at 100 oC until a semisolid product is formed. The product was put in a refrigerator until further use. [12]

Antiurolithias is activity

Method of crystal growth using compound microscope

Earlier reported method was used with some modifications. Synthetic urine supersaturated with calcium oxide was prepared at a constant temperature of 37˚C in capped vessels. The artificial urine was prepared immediately before use by mixing. For determination of the effects of plant extracts on crystal formation, preparation of the synthetic urine was performed in their presence at various concentrations.
The different stages of calcium oxalate monohydrate crystal growth were studied under a compound microscope in presence of different concentrations of the extract. Growth and aggregation patterns of calcium oxalate monohydrate crystals were observed and will be recorded.

Nucleation Assay

The inhibitory activity of the extracts on the nucleation of calcium oxalate crystals was determined by a spectrophotometric assay. Crystallization was initiated by adding 100µl of 4 mM calcium chloride and 100µl of 50 mM sodium oxalate solutions to 0.5ml of human normal urine, both prepared in a buffer containing 0.5ml of 0.05 mM Tris buffer and 0.5ml of 0.15mM NaCl solution at pH 6.5 and 37°C and adjusted to volume by adding 1.5ml of distilled water. The rate of nucleation was determined by comparing the induction time of crystals (time of appearance of crystals that reached a critical size and thus became optically detectable) in the presence of the extract and that of the control with no extract.

The percentage inhibition was calculated by following formula: % Nucleation inhibition = [(^A Control - ^A test)/^A control] x 100. [5]

Aggregation assay

The rate of aggregation of the calcium oxalate crystals was determined by a spectrophotometric assay with slight modifications. The Calcium Oxalate Monohydrate (COM) crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate of 50 mM each. Both solutions were then equilibrated. The solutions were then cooled to 37°C and then evaporated. The COM crystals were then dissolved with 0.5ml of 0.05mM Tris buffer and 0.5ml of 0.15 mM NaCl solution at pH 6.5 to a final concentration of 1 mg/ml. Absorbance at 620 nm was recorded. The rate of aggregation was estimated by comparing the slope of turbidity in the presence of the extract against control.

The percentage aggregation inhibition was then calculated by comparing the turbidity in control and test groups using the following formula: % aggregation inhibition = [(ΔA Turbidity Control-ΔA Turbidity Test)/Turbidity Control] ×100. [5]

RESULTS

Assessment anti urolithiatic activity plant extract by crystals growth using compound microscope

Calcium oxalate crystals were seen growing after putting the sample under a compound microscope. Results are given in fig 3.3. The activity is dose-dependent and proven to show a better inhibiting activity than the standard drug (Himalaya cystone). The plant extract shows full inhibiting activity at 100µg, 200µg, and 300µg. Results are given in fig 3.3.1, 3.3.2, and 3.3.3 respectively. While the standard drug show full inhibiting activity at 300µg. Results at a dose of 100µg, 200µg, and 300µg are given in fig 3.3.4, 3.3.5, and 5.3.6 respectively.

Assessment of anti-urolithiatic activity by nucleation assay

The percent of inhibition of the selected plant was determined after submitting samples to UV to check the absorbance value of the sample at the range of 620nm and the values generated for the standard drug (cystone: Hymalia) as 54.7% for 100µg.the result for the test sample generated as 30.191%, 34.55%, 50.87, and 59.86% for 100µg, 200µg, 400µg and 600µg respectively. Result are given in table 3.1 and 3.1 and fig 3.1.

Assessment of anti-urolithiatic activity by aggregation assay

The percent of inhibition of the selected plant was determined after submitting samples to UV to check the absorbance value of the sample at the range of 620nm and the values generated for the standard drug (cystone: Hymalia) as 63.1% for 100µg.the result for the test sample generated as 71.53%, 72.67%, 75.65, and 77.26% for 100µg, 200µg, 400µg and 600µg respectively. Results are given in table 3.2 and 3.2.1 and fig 3.2.

Assessment of IC50 value

A dose response curve was plotted to determine the IC50 values. IC50 values is defined as the concentration sufficient to obtain 50 % of a maximum scavenging capacity. The IC50 value and R2 value in the absorbance of nucleation assay of plant extract is found to be 116.6 and 0.9573 respectively. The IC50 value and R2 value in the absorbance of aggregation assay of plant extract is found to be 20.23 and 0.9967 respectively. Results are indicated in fig 3.4 and fig 3.5.

Table 1: Inhibition by nucleation assay.

Samples	Concentration(µg)	Trial 1	Trail 2	Trial 3	Trial 4	Mean value ± S.D
	Control	1.147	1.139	1.148	1.149	1.146 ± 0.005
Plant extract	100 µg	0.83	0.79	0.8	0.78	0.800 ± 0.022
	200 µg	0.76	0.75	0.76	0.73	0.750 ± 0.014
	400 µg	0.59	0.58	0.56	0.52	0.563 ± 0.031

	600 µg	0.47	0.46	0.45	0.45	0.460 ± 0.012
Cystone-Himalaya (std drug)	100 µg	0.53	0.52	0.5	0.51	0.515 ± 0.013

Values are expressed as mean ± SD.

Table 2: Percent of inhibition by nucleation assay.

Concentration	100 µg	200 µg	300 µg	400 µg	Mean ± S.D
Inhibition by Plant extract (%)	30.191	34.55	50.87	59.86	43.686 ± 13.888
Inhibition by Cystone (std drug)%	54.7	-	-	-	54.7 ± 0.014

Values are expressed as mean ± SD.

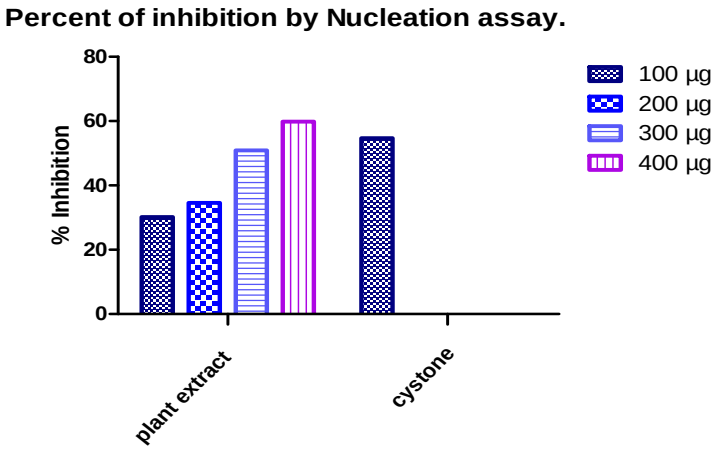

Figure 3: Percent of inhibition of plant extract and cystone drug by Nucleation assay.

Table 3: Inhibition by aggregation assay.

Samples	Concentration(µg)	Trial 1	Trail 2	Trial 3	Trial 4	Mean value ± S.D
	Control	1.6	1.701	1.702	1.703	1.676 ± 0.051
Plant extract	100µg	0.48	0.476	0.4733	0.4799	0.477 ± 0.003
	200µg	0.466	0.432	0.473	0.461	0.458 ± 0.018
	400µg	0.41	0.4112	0.4009	0.411	0.408 ± 0.005
	600µg	0.38	0.3823	0.3831	0.38	0.381 ± 0.002
Cystone-Himalaya (std drug)	100µg	0.61	0.64	0.59	0.63	0.618 ± 0.022

Values are expressed as mean ± SD.

Table 4: Percent of inhibition by aggregation assay.

Concentration	100µg	200µg	300µg	400µg	Mean ± S.D
Inhibition by Plant extract (%)	71.53	72.67	75.65	77.26	74.277 ± 2.64
Inhibition by Cystone(std drug)%	63.1	-	-	-	63.1 ± 0.000

Values are expressed as mean ± SD.

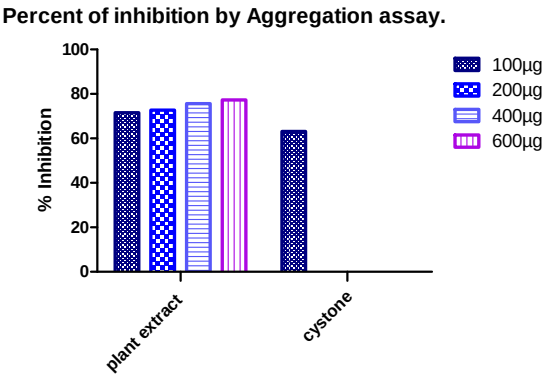

Figure 2: Percent of inhibition plant extract and cystone by aggregation assay.

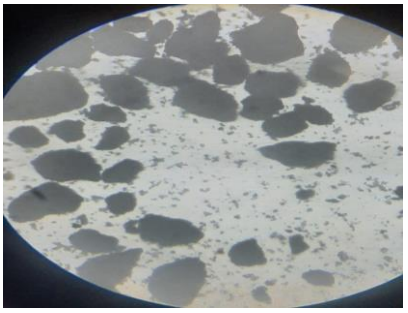

Figure 3: Calcium oxalate crystal growth.

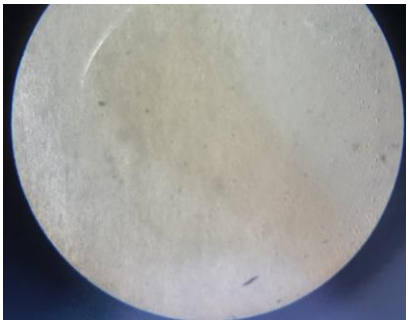

Figure 4: Anti-Urolithiatic activity of selected plant extract at 100 µg.

Figure 5: Anti-Urolithiatic activity of selected plant extract at 200 µg.

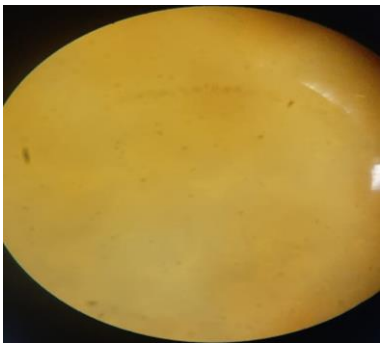

Figure 6: Anti-Urolithiatic activity of selected plant extract at 300 µg.

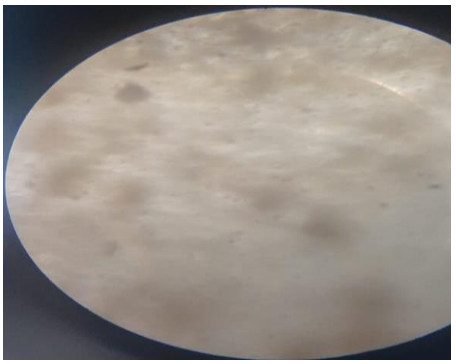

Figure 7: Anti-Urolithiatic activity of the standard drug (Himalaya Cystone) at 100 µg.

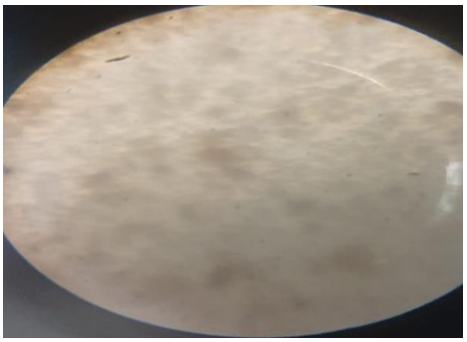

Figure 8: Anti-Urolithiatic activity of the standard drug (Himalaya Cystone) at 200 µg.

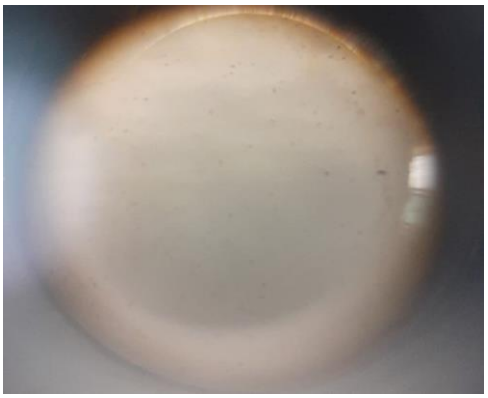

Figure 9: Anti-Urolithiatic activity of the standard drug (Himalaya Cystone) at 300 µg.

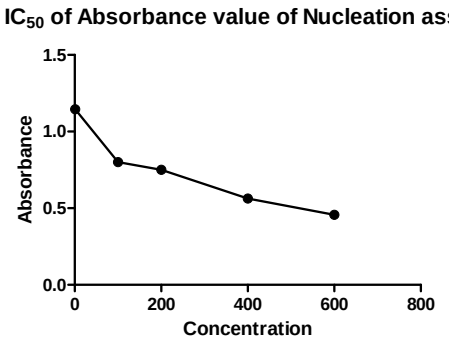

Figure 10: IC50 0f absorbance value of nucleation assay at different concentration.

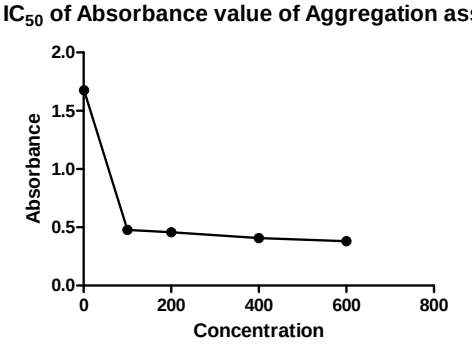

Figure 11: IC50 of absorbance value of aggregation assay at different concentration.

DISCUSSION AND CONCLUSION

The assessment of the anti-urolithiatic activity of selected plant extract by growing crystals under the compound microscope method had proved that MEBO showed inhibition of calcium oxalate crystal growth in a dose-dependent manner ($P< 0.05$).

The assessment of the anti-urolithiatic activity of selected plant extract by nucleation assay method had revealed that MEBO showed the inhibition of calcium oxalate crystal in a dose-dependent manner.

The assessment of the anti-urolithiatic activity of selected plant extract by aggregation assay method had revealed that MEBO showed the inhibition of calcium oxalate crystal in a dose-dependent manner.

Finally, we can say that the selected plant extract of Brassica oleracea L.var capitata (MEBO) showed *in-vitro* anti-urolithiatic activity. Based on these findings further investigations can be carried related to toxicity and *In-Vivo* anti-urolithiatic studies. The present findings gives preliminary evidence based support of anti-urolithiatic potential of Brassica oleracea L.var capitata.

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