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RESEARCH ARTICLE

Preliminary Phytochemical Screening and Evaluation of Antiurolithiatic Activity in Crude Extracts of *Cucumis Sativus*, *Citrullus Lanatus* and *Tribulus Terrestris*

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ABSTRACT

Aim: Herbal therapies had become integral part of health care sciences. Urolithiasis is a condition in which crystals in urine combines to form stones. The study evaluates antiurolithiatic activity of different extracts of the plants *Cucumis sativus*, *Citrullus lanatus* and *Tribulus terrestris*. In addition to that preliminary phytochemical screening of plants extract showed the presence of several phytochemicals. **Method:** Invitro antiurolithiatic activity can be studied by preparing calcium oxalate crystals by homogenous precipitation method and studying the effect of plant extracts on their ability to dissolve these crystals. In the present study, undissolved calcium oxalate was estimated by Kramer and Tisdall method. A total of 8 semi permeable membranes were taken and exactly 10 mg of calcium oxalate and 100mg of various drug extracts are packed in semipermeable membrane separately and this is allowed to suspend in a flask containing 100ml of 0.1M TRIS buffer. The contents remaining in the semi permeable membrane were titrated with KMNO₄ till a light pink colour end point was obtained by adding 2ml of 1N sulphuric acid. The result obtained are compared for activity with standard drug Tamsulosin hydrochloride (0.2mg). **Conclusion:** The study evaluates antiurolithiatic activity of different extracts of the plants along with phytochemical screening which resulted in presence of important phytochemical constituents like alkaloids, carbohydrates, fats and fixed oils, saponin glycosides, tannins, steroids and terpenoids. All aqueous, methanol, chloroform extracts of *Cucumis sativus*, *Citrullus lanatus* and *Tribulus terrestris* possessed significant activity in treatment of kidney stones.

Keywords: antiurolithiatic activity, phytochemical screening, Kramer and Tisdall method

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CONTENTS

1. Introduction	117
2. Materials and Methods.	117
3. Results and Discussion.	118
4. Conclusion.	119
5. References.	120

1. Introduction

Kidney stone disease (urolithiasis) is common worldwide disorder that has various pathophysiologies and etiologies. This condition is affecting populations from centuries of almost every region, culture, and race. Various demographic studies indicated that the rate of occurrence was altered by sex, age, and ethnicity. Urolithiasis affects people mostly in productive ages and is most commonly seen in males than females¹⁻³. Kidney stones may not or may show symptoms like severe pain with microscopic hematuria, ureteric obstruction, and urinary infection. In some cases symptoms also includes burning micturition, nausea, and vomiting, chills, fever, sick feeling, cloudy or smelly urine, increase frequency and urge to urinate⁴.

Super saturation plays primary role for precipitation, nucleation, aggregation, and eventually formation of kidney stone. The important factors that increase the risk of calcium oxalate stone formation in humans are hyperoxaluria, hypercalciuria, hyperphosphatemia, hypocitraturia, hyperuricosuria, hypomagnesiuria, low urinary pH, and low urinary output. Literature survey revealed the importance of phytotherapeutic agents as a replacement therapy for the treatment of urolithiasis.

Traditionally, *Cucumis sativus*, *Citrullus lanatus*, *Tribulus terrestris* has special importance as plants show protective properties in kidney diseases and have the ability to clear urine. Current study aims to support their traditional use in kidney disease using various biochemical parameters. The study investigates and compares the *in vitro* anti-urolithiatic activity between standard drug and the plant extracts from these selected sources⁵⁻⁶.

Complications of renal obstructions: Infectious diseases like pyelonephritis, Urinary extravastion, Fistula formation, renal insufficiency or chronic kidney disease, Bladder dysfunction, Pain.

Medical therapy: Doctors generally prescribe diuretics, such as furosemide to control hypercalciuria, and thus prevent calcium stones. They decrease the amount of calcium released by the kidneys into the urine by favoring calcium retention in bone. But when the use these diuretics sodium intake must be low⁷.

Cucumis sativus:

(Garden cucumber) belongs to Cucurbitaceae family. It is pale green in color with many seeds inside the fruit, which are pointed at both the ends. It is used as vegetable. The plant possesses various constituents like Alkaloids, glycosides, steroids, saponin, tannin, carbohydrates are present. Pharmacological analysis has revealed the presence of Anti-helminthic, diuretic, preparation of cosmetic products, used in treatment of skin inflammation and skin disorders⁸.

Citrullus lanatus (Water melon) belongs to Cucurbitaceae family. Creeping annual with deeply incised leaves and large round fleshy fruits in which are embedded small flat seeds. Grows in sandy soils. The plant possesses various constituents of nutritional value, such as, Lycopene, vitamin A, C, arginine, potassium, beta carotene, and anti-oxidants International Journal of Pharmacy and Natural Medicines

are present. Pharmacological analysis has revealed that used in treatment of Cancer, reduces the heart risk, BP, anemia, to prevent calculi formation⁹.

Tribulus Terrestris: (Land –calotrops) belongs to Zygophyllaceae family. Fruitare woody burr about 1 cm diameter with sharp spines to 6 mm long. Burr consists of 5 wedge shaped segments. Each segment has 2 unequal pairs of spines. It various constituents like Alkaloids, glycosides, steroids, tannin, carbohydrates, resins are present. Used to improve the Muscle growth, body strength, increases the number and motility of spermatozoa, and increases LH, Testosterone levels¹⁰.

2. Materials and Methods

The plant material *Cucumis sativus*, *Citrullus lanatus* and *Tribulus terrestris* were purchased from the nearby market. The samples were authenticated in the Department of Botany, P.B.Siddhartha College Of Technical Education, Vijayawada and a specimen copy of these drugs (Cs/s/2012;Cl/s/2012;Tt/f/2012) were stored in K.V.S.R. Siddhartha College of Pharmaceutical Sciences, Vijayawada for future reference. All the chemicals and reagents used in the present study were of analytical grade. Chloroform (Merck specialities pvt. Ltd.), Methanol (Loba chemicals) Benzene (Qualizens chemicals), Ammonia (Loba chemicals), Calcium chloride dihydrate (Hi-pure chemicals), Sodium oxalate (Finar chemicals), Ammonia (SD chemicals), Tris Buffer (SRL chemicals), Tamsulosin Hydrochloride (Flomax) Potassium permanganate (Loba chemicals)

Extraction¹²⁻¹⁴:

The seeds of *Cucumis sativus*, *Citrullus lanatus* and the fruits of *Tribulus terrestris* were subjected to sun drying in aluminum trays. They were spread evenly and allowed to dry for a period of two weeks continuously. The dried material is subjected to grinding process using household grinder and made into a coarse powder. The powdered plant material (*Cucumis sativus*, *Citrullus lanatus* and *Tribulus terrestris*) was subjected to maceration by successive solvent extraction using chloroform and methanol. The aqueous extract is obtained using soxhlet extraction for 24hrs. After extraction the contents were filtered by applying vacuum and stored in a desiccator. The samples were labeled and stored in a refrigerator until use. Yield was calculated and shown in table 1.

Preliminary Phytochemical Screening:

The results of phytochemical screening are displayed in table 2.

Evaluation Of *In vitro* Antiurolithiatic Activity

Preparation of Calcium oxalate crystals by homogenous precipitation¹⁸⁻¹⁹:

Equimolar solutions of calcium chloride dihydrate (A.R) dissolved in distilled water and sodium oxalate (A.R) dissolved in 10 ml of 2N H₂SO₄ and distilled water were prepared. Sufficient quantity was allowed to react in a beaker with slight aid of heat until more precipitation occurs. The resulting precipitate was calcium oxalate which was freed from traces of sulphuric acid by treating with

ammonia solution. Finally it was washed with distill water and dried at temperature 60°C for 4 hrs.

Preparation of the semi permeable membrane from farm eggs²⁰⁻²¹:

The semi permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin and yolk. Shell was removed chemically by placing the eggs in 2M HCl for two days which caused complete decalcification. Further, washed with distilled water and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Washed thoroughly with distilled water, and placed in ammonia solution, and then rinsed it with distill water. And finally it is stored in refrigerator at a pH of 7-7.4.

Invitro antiurolithiatic activity: The activity can be studied by preparing calcium oxalate stones and studying the effect of plant extracts on their ability to decrease (dissolve) the amount of calcium oxalate. In the present study undissolved Calcium oxalate was estimated by Kramer and Tisdall method¹⁶⁻¹⁷.

Standard Drug: Tamsulosin Hydrochloride – 0.2 mg

Preparation of 100 MI Of 0.1m Tris Buffer:

TRIS has a pKa of 8.1. So TRIS buffer is made at pH 7 used to prevent large swings in pH of solution.

Procedure: A total of 8 semi permeable membranes were prepared. Exactly 10 mg of calcium oxalate and 100 mg of various drug extracts and standard were weighed and packed in semi permeable membrane separately and carefully sutured. This was allowed to suspend in a flask containing 100 ml of 0.1M TRIS buffer. All the conical flasks were maintained at room temperature undisturbed for 7-8 hours. The contents remaining in the semi permeable membrane were transferred into a test tube. 2ml of 1N sulphuric acid was added and titrated with KMnO₄ till a light pink color end point obtained. The results were displayed in the tableno 3.

Calculation

Percent dissolved: Percent of mineral phase dissolved in the presence of test sample was calculated as:

$$\% \text{ Dissolved} = [(C-T)/C] * 100$$

Where,

C=precipitate of calcium oxalate remained in control

T= precipitate of calcium oxalate remained when test solution is used.

Equivalent factor: 1 ml of 0.9494N KMnO₄ equivalent to 0.1898 mg of Calcium.

3. Results and Discussion

Highest %yield was obtained with *Citullus lanatus* followed by *Cucumis sativus* chloroform extracts. Among the alcohol extracts, highest yield was obtained from *Tribulus terrestris* followed by *Cucumis sativus* and *Citrullus lanatus*. The highest yield of aqueous extracts was obtained from *Cucumis sativus* followed by *Citrullus*

lanatus and *Tribulus terrestris*. Yield confirms the presence of different active principles in the crude extracts. Non-polor constituents are found in chloroform extracts whereas alcohol and aqueous extracts possess polar constituents.

Cucumis sativus was found to contain alkaloids, carbohydrates, proteins, fats and fixed oils, saponins, steroids and terpenoids, flavanoids. *Citrullus lanatus* was found to contain alkaloids, carbohydrates, proteins, fats and fixed oils, saponin glycosides, tannins, steroids and terpenoids, flavanoids. *Tribulus terrestris* was found to contain alkaloids, carbohydrates, fats and fixed oils, saponin glycosides, tannins, steroids and terpenoids.

The ability of the tested extracts to dissolve calcium oxalate crystals under *invitro* conditions is evaluated. Among the tested extracts, *Citrullus lanatus* aqueous extract possessed significant anti urolithiatic activity with a %calcium oxalate dissolved of 91.5% as compared to standard drug, Tamsulosin 90.9%. The %calcium oxalate dissolved by *Tribulus terrestris* methanol extract was 84.6% which is slightly higher than *Cucumis sativus* aqueous extract ie 84.4%. *Citrullus lanatus* methanol extract dissolved 74.6% of the calcium oxalate which is the least value among the tested extracts. The increasing order of the ability to dissolve *invitro* renal stones is as follows CLM<TTA<CSM<CSA<TTM<CLA.

Tamsulosin acts as a selective alpha receptor antagonist. When these receptors in the bladder neck and Prostate gland are blocked, smooth muscle relaxation occurs resulting in less resistance to the flow of urine. Due to this, the symptoms associated with Benign Prostate Hyperplasia, urinary urgency, hesitancy and nocturia can be reduced. The exact mechanism by which these extracts possess anti urolithiatic effect is not clear but it may be similar to the standard drug to some extent.

Anti Urolithiatic Activity:

The aqueous extract of the drug *Citrullus lanatus* was found to possess significant antiurolithiatic activity when compared to other extracts. The activity was comparable with the standard drug Tamsulosin hydrochloride-0.2mg. The results are shown in table: 3.

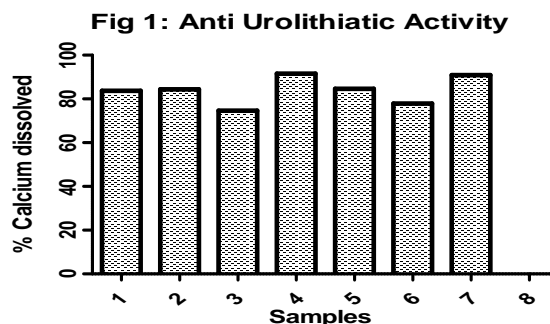


Figure 1: Anti urolithiatic activity

Table 1: Extracts obtained were labeled as follows

Abbreviation	Details
CSCE	<i>Cucumis sativus</i> chloroform extract
CSME	<i>Cucumis sativus</i> methanol extract

CSAE	<i>Cucumis sativus</i> aqueous extract
CLCE	<i>Citrullus lanatus</i> chloroform extract
CLME	<i>Citrullus lanatus</i> methanol extract
CLAE	<i>Citrullus lanatus</i> aqueous extract
TTCE	<i>Tribulus terrestris</i> chloroform extract
TTME	<i>Tribulus terrestris</i> methanol extract
TTAE	<i>Tribulus terrestris</i> aqueous extract

Table 2:% Yield of various extracts

Extract name	<i>Cucumis sativus</i>	<i>Citrullus lanatus</i>	<i>Tribulus terrestris</i>
	Yield%W/W		
Chloroform Extract	17.94%	22.53%	0.8%
Methanol Extract	2.6%	1.53%	5.03%
Aqueous Extract	9.06%	8.34%	1.59%

Table 3:Phytochemical screening of samples

Name of The Test	<i>Cucumis sativus</i>			<i>Citrullus lanatus</i>			<i>Tribulus terrestris</i>		
	CSCE	CSME	CSAE	CLCE	CLME	CLAE	TTCE	TTME	TTAE
DRAGENDORFF'S TEST	+	+	+	+	+	+	-	+	+
MAYERS TEST	+	+	+	+	+	+	-	+	+
WAGNER'S TEST	+	+	+	+	+	+	-	+	+
HAGER'S TEST	+	+	+	+	+	+	-	+	+
MOLISCH TEST	+	+	+	+	-	+	-	+	+
BIURET TEST	-	+	-	-	+	-	-	-	+
STERIODS AND TERPENOIDS	+	+	-	+	+	+	+	+	-
FLAVANOIDS	-	+	-	-	+	-	-	-	-
ANTHRAQUINONE GLYCOSIDES	-	-	-	-	-	-	-	-	-
SAPONIN GLYCOSIDES	-	+	-	-	+	-	-	+	+
TANNINS	-	-	-	-	-	+	-	+	+

+ indicates presence

- indicates absence

Table 4:Antiuroliathatic activity of the extracts

S.No	Group	Vol. Of KMnO ₄ Consumed 0.9494N	Amount of Calcium Oxalate Estimated	Amount Of Calcium Oxalate Dissolved	% Dissolved
1	CSME	1.36	0.25	9.75	83.7
2	CSAE	1.4	0.24	9.76	84.4
3	CLME	2.2	0.39	9.60	74.6
4	CLAE	0.8	0.13	9.87	91.5
5	TTME	1.25	0.237	9.77	84.6
6	TTAE	1.7	0.34	9.66	77.9
7	STANDARD	0.765	0.14	9.85	90.9
8	CONTROL	8.5	1.54	8.45	0

4. Conclusion

It is evident from the literature that renal stones are one of the risk factors which may be fatal if left untreated. The study evaluates antiuroliathatic activity of different extracts of the plants *Cucumis sativus*, *Citrullus lanatus* and *Tribulus terrestris*. From the preliminary phytochemical screening it is evident that *Cucumis sativus*, *Citrullus lanatus*, *Tribulus terrestris*, were found to contain important phytoconstituents like alkaloids, carbohydrates, fats and fixed oils, saponin glycosides, tannins, steroids and

terpenoids. All aqueous and methanol extracts of *Cucumis sativus*, *Citrullus lanatus* and *Tribulus terrestris* possessed significant activity. This has provided primary evidence for the use of *Cucumis sativus*, *Citrullus lanatus*, *Tribulus terrestris* in treatment of kidney stones. The crude aqueous extract isolated from *Citrullus lanatus* produced highest dissolution of calcium oxalate stones in comparison to *Cucumis sativus* and *Tribulus terrestris* extracts. Its activity is comparable with that of the standard drug Tamsulosin hydrochloride-0.2mg. This *in vitro* study has given a very valuable data and showed that these extracts possess quite

promising potential antiurolithiatic activity. Further, *in vivo* studies are required to strengthen the work and prove their therapeutic usefulness.

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