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Evaluation of in-vitro anti-urolithiatic activity of ethanolic extract of *sterculia urens*

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Abstract

All individuals have traditionally considered medicinal plants as a healthy source of life, and its therapeutic characteristics have proven to be quite effective in treating a wide range of illnesses. Another benefit of using medicinal plants is that they are completely natural. Since ancient times, people have utilized traditionally to treat common infectious disorders, and the belief that some plants possessed therapeutic properties was widely recognized even before mankind discovered there were microorganisms. Due to their antibacterial qualities, several plants have been utilised in traditional medicine for a long time. The therapeutic benefit of these plants is specifically found in a few chemical compounds that have a clear physiological effect on the human or animal body. Alkaloids, flavonoids, tannins, and phenolic compounds are among these bioactive ingredients, which are primarily secondary metabolites, that are most significant. Cells of microorganisms are poisoned by these phytochemicals. Individuals with Nephrolithiasis or kidney stone disease, is a condition that accumulate calculi (stones) within the renal pelvis and tubular lumens. Stones are formed when crystals precipitate (separate) from urine. When the urine concentration of compounds that help prevent stone formation (citrate) is low or when that of substances that help create crystals (calcium, oxalate, and uric acid) is high, stones may form. Current research aims for assessment of the invitro anti urolithiatic activity of *Sterculia urens* by employing the method such as nucleation assay Our study indicates that, sterculia urens can be a great source of antiurolithiatic agents, which are most likely mediated by the suppression of CaOx crystallization.

Keywords: Anti-urolithiatic activity, *sterculia urens,* Herbal formulations.

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1. Introduction

Only urinary tract infections and pathological diseases of the prostate are more prevalent urinary tract ailments than urinary stones (BPH and prostate cancer). Since ancient times, the ailment known as "stone disease" has been a serious issue for the human population. The Aphorisms of Hipparchus and even Hippocrates have early observations of the illness [1]. With an estimated population prevalence of 2-3% and an estimated prevalence lifetime risk of 12% for white males and 5-6% for white females, the disease is extremely in both men and women. The recurrence rate over a life time is about 50%. About 10% of recurrences occur within a year, 35% within five years, 50% within ten years, depending on the interval between recurrences. The interval between recurrences is variable, with approximately 10% within one year, 35% within five years, and 50% within 10 years [2].

Individuals with Nephrolithiasis or kidney stone disease, is a condition that accumulate calculi (stones) within the renal pelvis and tubular lumens. Stones are formed when crystals precipitate (separate) from urine. When the urine concentration of compounds that help prevent stone formation (citrate) is low or when that of substances that help create crystals (calcium, oxalate, and uric acid) is high, stones may form [3]. Urinary stone problems are a widespread problem that affects humans, animals, and even birds. In epidemiological investigations of urolithiasis, the terms incidence, prevalence, and lifetime prevalence are typically used. In the majority of cases, urolithiasis is recurring once it has been diagnosed. After the first stone event, the recurrence rates are 14%, 35%, and 52% at 1, 5, and 10 years, respectively. Within 10 years, urinary calculi that have previously occurred in more or less 50% of patients reappear. According to a recent study, the recurrence rates are expected to be 10% annually, totaling 50% after a period of 5 to 10 years, and 75% over a period of 20 years [4].

Although the highest risks of 20.1% have been reported in Saudi Arabia, the probability of acquiring urinary calculi in adults appears to be more prevalent in the western hemisphere than the eastern hemisphere. Urolithiasis incidence rates have been found to be 5-9% in Europe, 12% in Canada, and 13-15% in the USA. Due to the increased risk of dehydration in hot temperatures, the incidence rate in the Middle East rises to 20-25%. 5.4% of people worldwide are predicted to acquire a urinary calculus at least once in their lifetime, according to data from a national survey on urolithiasis conducted in Japan between 1965 and 1987. Some regions have those alarming occurrences that they are referred to as stone belts. British islands, Scandinavian nations, Central Europe, Northern Australia, and Mediterranean nations are among the regions where urinary calculi are more common [5].

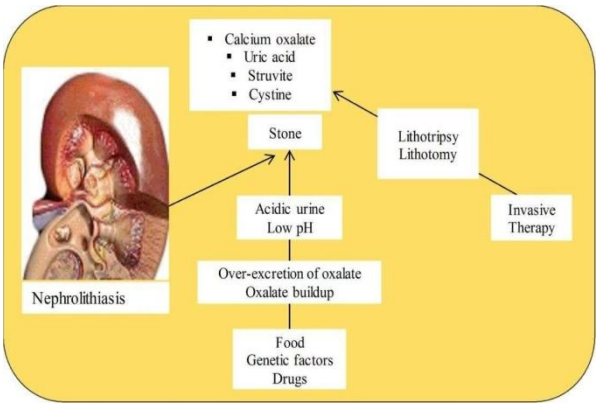

Figure 1. Nephrolithiasis reasons and treatments.

World wide interest in natural products as preventive and therapeutic agents as led a greater appreciation of the rich heritage of traditional of medicine. The selection of scientific and systemic approach for the biological evalution of plant world onthe basis of their use in the traditional system of medicine forms a basis for art ideal approach in the development of the development of the new drugs from plants. A plant species called Sterculia urens, which is native to India and has been brought to Burma, is a member of the Malvaceae family. A small to medium-sized tree, also known as the "bhuty" in Marathi, "kulu," "Indian tragacanth," "gum karaya," "gum katira," "sterculia gum," or "kateera gum," that has a light-colored trunk. The present aim to evaluate the anti urolithiatic of *Sterculia urens* leaves extract [6].

2. Materials:

In the present study, the leaves of *sterculia urens* were collected from local areas of Tirupathi and authenticated by Prof. Madhav chetty, Department of Botany, Sri Venkateswara University, Tirupathi, Andhra Pradesh. The authenticated leaves of *sterculia urens* were air dried and subjected to size reduction to get coarse powder and was subjected to standardization with differentparameters.

3. Methodology

3.1. Preparation of ethanolic extract

After being washed, the plant material was dried in a hot air oven at a maximum temperature of 50° C and ground into a coarse powder. In the soxhlet apparatus, 150 gms of powdered material were subjected to solvent extraction with ethanol. The extraction process was continued until the solution was colorless, and then it was concentrated with a rotary evaporator under reduced pressure. The percentage yield was calculated from the dried extract and shown in experimental results [7].

Figure 1: Soxlhet extraction and distillation of *sterculia urens extract.*

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3.2. Qualitative phytochemical analysis

Understanding the wide range of chemical compounds produced by plants will be made easier with the use of qualitative phytochemical screening and quantitative analysis of those metabolites will aid in extraction, purification and identification of bioactive substances with potential human benefits [8]. In phytochemical evaluation, the powdered leaves were subjected to phytochemical screening for the identification of various plant constituents, defined for their potential bioactive compounds, which were separated and subjected to comprehensive structural analysis [9].

3.3. Qualitative chemical investigation:

To determine the various photo constituents, qualitative chemical tests were carried out on all of the leaf extracts from Sterculia urens [10]. The different procedures and used reagents are listed below , along with observations.

1) Test for Proteins

Preparation of Test Solution: The extract was dissolved in water to prepare the test solution.

a)Biuret test (Generaltest): To 3 ml T.S added 4% NaOH and few drops of 1% CuSO4, solutionobserved for violet or pink colour.

b) Millon'stest (forproteins)

Mixed 3 ml TS with 5 ml Millions, white precipitate obtained. It was reported that when precipitate is warmed, it either dissolves and turns brick red.

c) Xasthoproteintest (Forproteincontainingtyrosineortryptophan): Mixed 3ml T.S. with 1ml concentrated H2SO4, observed for white precipitate.

d) Precipitation test

With the reagents absolute alcohol, 5% HgCl2, 5% CuSO4, 5% lead acetate, and 5% ammonium sulphate, the test solution yielded a white colloidal precipitate.

2) Tests for Steroids:

Preparation of test extract solution: The extracts were re-fluxed separately until complete saponification using an alcoholic potassium hydroxide solution. Saponified extract was diluted with Water and unsaponifiable mutter win extracted with diethyl ether. By dissolving the rider in chloroform after the ethereal extract had been evaporated, the residue (unsaponifiable matter) was subjected to the following process.

a) Salkowski reaction: To 2 ml of extract, 2 ml chloroform and 2 ml concentrated H2SO4 was added. Shake well, whether chloroform layer appeared red and acid layer showed greenish yellow fluorescence was observed.

b) Libermann-Burchard test:mixed 2ml of extract with chloroform. Added 1-2 ml acetic anhydride and 2 drops concentrated H2SO4 from the side of test tube observed for first, then blueand finally green colour.

c) Libermann's test: Mixed 3 ml of extract with 3 ml of acetic anhydride. Heated and cooled. Added few drops of concentrated H2SO4, and observed for blue color

3) Tests for Amino Acids:

a) Ninhydrin test (General test): 3 ml T.S. and 3 drugs 5% Ninhydrin solution were heated in boiling water bath for 10 min. Observed for purple orbluish colour.

b) Test for Tyrosine: 3 ml T.S. and 3 drops Millons reagent. Solution observed for dark red colour.

c) Test for tryptophan: To 3 ml T.S. added few drops glyoxalic acid and concentrated H2SO4 observed for reddish violet ring at junction of the two layers.

4) Tests for Glycosides:

Preparation of test solution: The test solution was prepared by dissolving extract in the alcohol or hydro-alcoholicsolution.

5) Tests for Cardiac Glycosides:

a) Baljet'stest: A les solution observed for yellow to orange colour with sodium picrate.

b) Brominewatertest: Test solution dissolved in bromine water giving yellow precipitate

c) Legal's test (For cardenoloids): To aqueous or alcoholictest solution, added 1 ml pyridine and 1 ml sodium nitroprusside observed for pink to red colour.

d) Test for deoxysugars (Kellar Killani test): To 2 ml extract added glacial acetic acid, one drop of 5% feCl3, and concentrated H2SO4 observed for reddish brown color at junction of the two liquid and upper layers bluish green.

e) Libermann's test (For bufadenolids): Mixed 3 ml extract with 3 ml acetic anhydride. Heated and cooled Added few drops concentrated H2SO4 observed for bluecolor

Test for Anthraquinone glycosides:

a) Test Modified Borntrager's test: C-glycosides of anthraquinones require more drastic conditions for hydrolysis. Hydrolysis of the drug was carried out with 5 ml of dilute HCI and 5 ml of 5% solution of FeCl3. For hydrolyzed extract procedure was carried out as described under Bontrager's test

b) Boratrager's test: Boiled powdered drug with 5

ml of 10% Sulphuric acid for 5 mins. Filtered while hot, cooled the filtrate shaken gently with equal volume of benzene. Benzene layer was separated and then treated with half of its volume solution of ammonia (10%) Allowed it to separate. The ammonical layer acquired rose pink color due to the presence of anthraquinone.

Cyanogenetic glycosides:

a) Grignard's test: Strips of sodium picrate filter paper were inserted between split cork stopper which was fitted in to the neck of the test tube containing a small amount of powdered drug in water and was exercised that the paper didn't touch the inner side of the test tube. The content was warmed for half an hour. The red color of the strips indicated the presence of cyanogenetic glycosides.

6) Tests for Alkaloids:

a) Dragendorff's test: To 2-3 ml filtrate added few drops of Dragendorff's reagent and observed for orange brown precipitate.

b) Mayer's test: 2-3 mi filtrate with few drops Mayer's reagent observed for precipitate.

c) Hager's test: 2-3 ml filtrate with Hager's reagent observed for yellow precipitate.

d) Wagner's test: 2-3 ml filtrate with few drops of Wagner's reagent and observed for yellow precipitate.

7) Tests for flavonoids- The flavonoids are all structurally derived from the parent substance called flavones. The flavonoids occur in the free form as well as bound to sugars as glycosides for this reason, when analyzing flavonoids, it is usually better to examine the flavonoids in hydrolyzedplant extracts

Preparation of test solution.

i.To a small amount of extract added equal volume of 2M HCL and heated in a test for 30 to40min. at 100C

i. The cooled extract was filtered, and extracted with ethyl acetate.

ii. The ethyl acetate extract was concentrated to dryness, and used to test for flavonoids

a) Shinoda test: To dried powder or extract, added 5 ml 99% ethanol, few drops of concentrated HCL and 0.5 g magnesium turnings Pink color was observed. The small quantity of residue, added lead acetate solution observed for yellow coloured precipitate. Addition of increasing amount of sodium hydroxide to the residue showed yellow coloration, which was decolorized after addition of acid was observed.

b) Ferric chloride test: Test solution, add few drops of ferric chloride solution observed forintense green color.

8) SaponinsPreparation of test solution: The test solution was prepared by dissolving extract in the water.

a) Foam test: Test solution when shaken showed the formation of foam, which was stable for atleast 15 min.

b) Haemolysis test: 2 ml of 18% sodium chloride in 2 test tubes was taken, to one test tube added distilled water and to other 2 ml test solution. Few drops of blood were added to both the test tubes. Mixed and observed for haemolysis under microscope.

c) Test for steroidal saponins: The extract was hydrolyzed with dilute Sulphuric acid and extracted with chloroform. The chloroform layer was tested for sterols.

d) Test for triterpenoid saponins: The extract was hydrolyzed with dilute Sulphuric acid and Extracted with chloroform. The chloroform layer was tested for triterpenoids.

9) Tannins and Phenolic compounds: To 2-3 ml of alcoholic or aqueous extract, added few drops of following reagents:

a) 5% FeCl3 solution: Deep blue-black color.
b) Lead acetate solution: White precipitate.
c) Bromine water: Discoloration of bromine water.
d) Acetic acid solution: Red color solution
e) Dilute iodine solution: Transient red color.

3.4. In-vitro anti-urolithiatic activity:

Method: Nucleation assay

Procedure

Effect of sterculia urens calcium oxalate(CaOx) crystal formation was determined by means of nucleation assay. Calcium chloride (CaCl2)(5mmol/l)and sodium oxalate (Na2C2O4) solution (7.5mmol/l) were prepared in Tris-HCL (0.05 mol/l) and NACL (0.15mol/l) buffer (PH 6.5). Dilutions of sterculia urens rainging from μg/ml were prepared in distilled water [11-14].

One millimeter of each sterculia urens concentration was mixed with 3 ml CaCl2 solution followed by the addition of 3 ml NaC2O4 solution. Final mixtures were incubated for 30 min at 37ºC . The optical density (OD) of the mixtures was then measured at 205 nm wave length. Percent inhibition by sterculia urens was calculated using under mentioned formula and compared to that calculated for the standard polyherbal drug, cystone.

$$\% \text{ Inhibition} = 1 - \frac{\text{ODTest}}{\text{OD Control}} \times 100$$

Preparation of Cystone Solution

100mg of standard cystone powder was dissolved in 100ml of water in a 100ml of volumetric flask then adjust the volume up to 100ml then 1ml,2ml,4ml,6ml,8ml,10ml of this solution was diluted to 10ml of diluted concentration of solution to this solution was 10µg,20µg,40µg,60µg,80µg,100µg.

3.5. Statistical Analysis

Qualitative results of all experiment performed in triplicates were experssedas mean± S.E.M (Standard Error of mean). Statistical Computation were Performed on Graphpad prism 8 software.

4. Results and Discussion

The malvaceae family member Sterculia urens, which has medicinally significant bioactive ingredients, is evaluated in the current study with a focus on its biological activity.The plant material was air dried and reduced to coarse powder the powder material was subjected to solvent extraction in soxhlet apparatus with ethanol. The sox halation was continued and until the colorless solution was obtained and the solution was concentrated in rotary evaporator and reduced pressure and yield of extract is 10.625 g the colour of extract was greenish black in colour with characteristic taste.

CaOx is the principal component of the majority of stones in renal calculus, which is made up of mucopolysaccharide, urates, CaOx, calcium phosphate, and calcium carbonate. The recurring stone in the medical treatment of urolithiasis, is a very severe concern. In order to reduce the recurrence of hypercalciuria and hyperoxaluria, medications such thiazide as a diuretic and alkali-citrate are employed. These medications are thought to be less effective than other options.

Over time, more focus has been placed on herbal plants in search of potent anti-urolithic drugs due to the intricacy of lithogenesis and high risk of kidney stone recurrence. Herbal anti-urolithiatic medications have shown more promise because they are affordable and have minimal to no adverse effects. The objective of the current research is to examine these significant CaOx stone formation events and the effectiveness of Sterculia urens as an anti-urolithiatic.

In the present investigation, evaluated in vitro antiurolithiatic activity of test drug in nucleation assay methods. The reaction between calcium chloride and ammonium oxalate resulted in the initiation of nucleation of CaOx crystals which is in accordance with previously reported studies.

The pathophysiology of CaOx urolithiasis requires nucleation. In essence, nucleation describes a thermodynamically induced phase transition in which dissolved materials in a supersaturated solution spontaneously crystallise. While performing the nucleation assay, a similar phase change and CaOx crystal formation were observed.Significant inhibition in the nucleation of CaOx crystals was observed in the presence of Sterculia urens which was slightly lower than in the presence of Cystone. This suggests the anti crystallization activity of Sterculia urens against CaOx crystallization. One possible mechanism of anticrystallization activity of Sterculia urens could be its abilityto complex with free calcium and oxalate ions, thus preventing the formation of CaOx complexes, as has also been suggested for *Sarghassumwightti.*

Literature of the previous studies demonstrated that Sterculia urens exerts an anti- urothiatic effect in numerous chronic inflammatory diseases and concluded that Sterculia urens as an extremely potent anti-urolithaitic molecule. So, it is another more beneficial action to reduce inflammation due to kidney stones formation. Finally, the results of the present investigations suggest that the Sterculia urens has in-vitro anti- urolithiatic effect on CaOx crystals.

TABLE NO 1: Macroscopic Characteristics of Leaves of *Sterculia Urens*

S.NO	Parameters (physical test)	Observation of flower
1.	Texture	Smooth
2.	Colour	Green
3.	Odour	Charateristic
4.	Taste	Characteristic

TABLE NO 2: Analysis of extract of leaves of *Sterculia urens*

S.NO	Extract	Colour
1.	Ethanol	Browish

TABLE NO 3: Qualitative phytochemical analysis of Ethanolic Exatract of *sterculia urens.*

S.NO	CHEMICAL CONSTITUENTS	ETHANOLIC EXTRACT
1.	Alkaloids	+
2.	Reducing sugars	+
3.	Glycosides	+
4.	Cardiac glycosides	+
5.	Saponin glycosides	+
6.	Flavonoids	+
7.	Steroids	+
8.	Tannins	+

Table No 4: Evaluation for Antiurolithiatic Activity by Nucleation Assay

S.No.	Substance	Concentration (µg/ml)	Absorbance	% Inhibition
1	Control	-------	0.407±0.009	-------
2	Sterculia urens	100	76.333±0.231	42.190±0.894
		200	79.600±0.400	57.963±1.055
		400	82.533±0.503	62.307±0.30
		600	85.33±0.0577	72.797±0.235
		800	89.400±0.173	81.50±0.50
		1000	92.300±0.300	91.200±0.346
3	Cystone	100	71.48±0.108	40.20±0.721
		200	75.343±0.510	56.533±0.503
		400	78.343±0.223	60.73±0643
		600	82.107±0.186	71.450±0.427
		800	84.567±2.055	79.833±0.764
		1000	88.510±0.689	88.130±0.848

All Values represent Mean±SEM; n=3

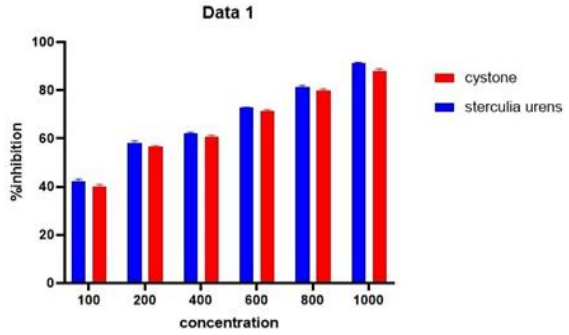

Figure 3. Showing Effect of Ethanol Extract of *sterculia urens* on cystone.

Conclusion

In study, an effort has been made to determine the sterculia urens plant's potential for treating conditions including urolithiasis. The leaves of the malvaceous plant sterculia urens were chosen for the study based on the literature review, and the following variables were investigated. The ethanolic extract of *sterculia urens* was identified for the presence of flavonoid, steroids terpenoids, alkaloids, reducing sugar, saponins, tannins. The presence of above mentioned phytochemical constituents may be responsible for anti urolithiatic activity. In developing countries, the incidence of renal stones is significantly increasing. Based on the data available in the literature Sterculia urens was selected. According to our study, sterculia urens can be a great source of antiurolithiatic agents, which are most likely mediated by the suppression of CaOx crystallization. It possesses strong free radical scavenging action as a flavonoid molecule, which would improve its utility to treat urolithiasis-induced oxidative stress. Moreover, in-vivo studies are required to reinforce the research and demonstrate its therapeutic value.

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