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Phytochemical and pharmacological evaluation of fruits of *Elaeocarpus serratus* for antiurolithiatic activity: An *In vitro* study

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ABSTRACT

Background

Medicinal plants are highly esteemed all over the world as a rich source of therapeutic agents for the prevention and treatment of various diseases. Since ages, herbs are being used for treating different ailments in different parts of world by different communities.

Objective

The present research work involves the evaluation of the pharmacognostical, phytochemical and *in vitro* antiurolithiatic activity of *Elaeocarpus serratus* fruit extract.

Method

The aqueous extract of *Elaeocarpus serratus* was prepared and phytochemical analysis indicated the presence of carbohydrate, phytosterols, tannins and phenolics. The semi-permeable membranes of eggs were used as dissolution bags. The spectrophotometric analysis has given good dissolution of calcium. Calcium oxalate was released 53.12% and 41.59% in case of standard and test respectively whereas calcium phosphate was released 55.72% and 32.06% for the standard and tests respectively.

Conclusion

So, it can be concluded that the extract of *Elaeocarpus serratus* has proved capability to dissolve calcium which may be helpful in removal of stone forming elements in urinary tract. Cystone, the standard drug has shown better demineralization for calcium phosphate as secondary for stone forming in urinary tract when compared to the extract of *Elaeocarpus serratus*.

Keywords: *Elaeocarpus serratus*, Calcium oxalate, Cystone, antiurolithiatic, Calcium phosphate

INTRODUCTION

Medicinal plants are the major parts of traditional systems in developing countries. Till today the herbal medicine maintains health and to prevent, alleviate, or cure diseases and shows a positive impact on therapeutic remedies in many

developing countries [1]. Urolithiasis is referred to most common disorder of the urinary tract. It affects about 10-30% of the population in industrialized countries. It occurs more frequently in men than women but rare in children [2]. Kidney stones typically form in the kidney, and leave the

body in the urine stream. Kidney stone can be caused when substances in the urine, such as calcium, oxalate, and phosphorus become highly concentrated. Calcium is one of the component of most common type of human kidney stones. The vitamin C and D supplements has a high increased rate of calcium oxalate formation. The organic matrix of kidney stone consists of glycosaminoglycans (GAG's), lipids, carbohydrates and proteins. The stone matrix includes proteins, non-amino sugars, glucosamine, water and inorganic ash. The matrix contains phospholipids of the total lipid, which promote the formation of calcium oxalate and calcium phosphate. Albumin is the major component of the matrix of all the stone types. Brushite stone is a hard phosphate mineral with an increasing incidence rate and is resistant to shock wave and ultrasonic lithotripsy treatment [3]. Kidney stones includes calcium containing stones, struvite stones, uric acid stones, cystine stones, drug induced stones. Calcium oxalate crystals in urine appear as 'envelopes' microscopically. The main constituent of calcium stones is brushite i.e. calcium hydrogen phosphate [4]. It occurs in the circumstances when the urine is alkaline i.e. pH more than 5.5. Calcium oxalate crystal formation is also one of the toxic effects of ethylene glycol poisoning [5]. It is also known as magnesium ammonium phosphate stones. Struvite stones form most often in the presence of infection by urea splitting bacteria [6, 7]. Uric acid stones can form in people who don't drink enough fluids or who loses it, those who eat a high-protein diet, high in purines, and have gout certain metabolic abnormalities like obesity or certain genetic factors also may increase the risk of uric acid stones [8]. Cystine stones constitute about 3% of the urinary calculi. It occurs in the circumstances of rare genetic disorder [9]. Drug induced stones produce calculi may be divided into two groups. The first one includes poorly soluble drugs with high urine excretion that favour crystallization in the urine. The second group includes that provoke the formation of urinary calculi as a consequence of their metabolic effects on the urinary pH [10, 11]. Drugs such as guaifenesin, triamterene, atazanavir, and sulfa drugs induce these stones [12]. Kidney stone size influences

the rate of spontaneous stone passage. Management of pain often requires intravenous administration of NSAIDs or opioids [13, 14]. Medical expulsive therapy is defined as the use of medications to speed the spontaneous passage of stones in the ureter [15, 16]. Several agents like alpha adrenergic blockers and calcium channel blockers can be effective. Extracorporeal shock wave lithotripsy is defined as a non-invasive technique for the removal of kidney stones. It involves the use of a lithotripter to deliver externally applied, focused, high-intensity pulses of ultrasonic energy to cause the fragmentation of a stone over a period of around 60 minutes [17, 18, 19]. In the present study the fruits of *Elaeocarpus serratus* were investigated for its pharmacognostical, phytochemical and pharmacological activity, on the removal of kidney stone from the body.

MATERIALS AND METHODS

Chemicals and Reagents

All the reagents used were of analytical grade obtained from Sigma Chemical Co. St. Louis, USA and S. D. Fine Chemical Ltd., Mumbai. Cystone tablets were purchased from Himalaya Drug Company, Bengaluru.

Instruments and equipments

Standard glasswares of Borosil were used for experimental purpose. Hot air Oven, Muffle furnace, Water bath (Remi Instrument Ltd., Mumbai), Digital balance (CP225 D, Sartorius AG, Germany), Mixer grinder (Jaipan Instrument Ltd., Mumbai), Vacuum pump (Riviera Glass Pvt. Ltd., Mumbai), U.V. cabinets (Technology Instruments, Mumbai), Incubator (Eltek Equipment, Mumbai), UV spectrophotometer (UV-1800, Shimadzu, Japan) were used for the study.

Collection and Identification of Plant:

The fruit was collected in the month of August from Siliguri, West Bengal India and identified with the help of standard literature [20] authenticated by Dr. M. Chowdhury, Botanist, Dept. of Botany, North Bengal College and University, Siliguri, West Bengal. India.



Fig 1: Photographic image of *Elaeocarpus serratus* fruit parts

Extraction process

The powdered material of the fruit of *Elaeocarpus serratus* was successively extracted first with petroleum ether and then with water for 3-7 days by cold maceration technique [21, 22]. The petroleum ether extract was completely dried under reduced pressure in a rotary vacuum evaporator at 40°C temperature where as the aqueous extract was dried in a freeze drier [23]. Both the extracts were stored in vacuum desiccators for further use.

Physicochemical Screening

The various physicochemical parameters were studied as per standard protocols which include determination of ash contents (total ash, water soluble and acid insoluble), extractive values and moisture content.

Determination of Total ash

The fruit powder was taken in a silica crucible previously ignited and weighed. It was incinerated

by gradually increasing the heat not exceeding dull red heat (450°C) until free from carbon, cooled and weighed. The percentage of ash was calculated. The procedure was repeated five times to get constant weight.

Determination of water soluble ash

The Total ash was boiled with 25 ml of water for 5 minutes and was filtered through an ash less filter paper. It was followed by washing with hot water. The filter paper was ignited in the silica crucible, cooled and the water insoluble matter was weighed. The water soluble ash was calculated by subtracting the water insoluble matter from the total ash.

Determination of acid insoluble ash

The total ash obtained was boiled for 5 minutes with dilute hydrochloric acid and acid soluble ash was calculated as mentioned in above step.

$$\text{Ash Value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Determination of alcohol soluble extractive

2 gm of the air-dried coarse powder of *Elaeocarpus serratus* were with 100 ml of 90% ethanol in a closed flask for 24 hours. Then the flask is been frequently shaken during the first 6 hours and allowed to stand for 18 hours.

Thereafter, it was filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs.

$$\text{Extractive Value} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

Determination of water soluble extractives

The procedure adopted for water soluble extractive values was similar to that adopted for the determination of alcohol soluble extractive values.

Determination of moisture content

2 gm of the air dried crude drug was accurately weighed in a tarred watch glass. The drug was kept in hot air oven at 105°C and dried for a period until constant weight obtained. The difference in weight gives the moisture content of the drug [24 -27].

Fluorescent analysis

The fluorescent analysis of shade dried and powdered, petroleum ether extracted plant material of *Elaeocarpus serratus* was studied under UV light and daylight. Powder was subjected to different chemicals like 50% H₂SO₄, 50% HNO₃, 5% KOH, Methanol, 1N HCl, 1N Methanolic NaOH in methanol. The fluorescence analysis of these fruit extracts were observed under ordinary visible light and also under UV light (245 nm) [28, 29].

Qualitative phytochemical analysis

The petroleum ether and aqueous extract of fruits of *Elaeocarpus serratus* were subjected to the following chemical tests for the identification of various active constituents.

Detection of Carbohydrates

The extract was dissolved first in HCL (except for the methanolic extract) and then water and filtered. The filtrate was subjected to the following tests:

Molish's test

To 2-3 ml extract, few drops of α -naphthol solution in alcohol were added and concentrated H₂SO₄ was added from the sides of the test tube. A violet ring at the junction of two liquids indicates the presence of sugar.

Fehling's test

1ml Fehling's A and 1ml Fehling's B Solution was mixed and boiled for one minute. To this equal volume of test solution was added. On heating in boiling water bath for 5-10 min., initially it forms a yellow and then brick red precipitate.

Benedict's test

Equal volume of Benedict's reagent and test solution were mixed in test tube and heated on boiling water bath for 5 min. Solution appears green, yellow or red color depending on the amount of reducing sugar present in test solution.

Barfoed's test

Equal volume of Barfoed's reagent and test solution were added and heated on boiling water bath and cooled. Red precipitate was observed indicating the presence of monosaccharide.

Detection of Saponins

Foam test

The drug extract or dry powder was shaken vigorously with water. It was then observed for persistent foam.

Detection of Alkaloids

Dragendorff's test

To 2-3 ml filtrate, few drops of Dragendorff's reagent were added. Then it was observed for the formation of orange brown precipitate.

Mayer's test

2-3 ml of filtrate was mixed with few drops of Mayer's reagent then it was observed for the formation of precipitate.

Wagner's Reagent

2-3 ml filtrate was treated with Wagner's reagent then it was observed for the formation of reddish brown precipitate.

Detection of Tannins and Phenolic Compounds

To 2-3 ml of the filtrate, few drops of following reagents were added and the changes in color were observed:

- 5 % FeCl₃ solution: - deep blue- black color.
- Lead acetate solution: - white precipitate.

Detection of Flavonoids

Shinoda test

To the filtrate, 5 ml of 95% ethanol, few drops concentrated HCl and 0.5 g magnesium turnings were added. Pink color was observed. This indicates the presence of flavonoids.

Detection of Proteins and Amino acids

Biuret test

To 3 ml test solution, 4 % NaOH and few drops of 1 % CaSO₄ solution was added and observed for violet or pink colour.

Million's test

3 ml of test solution was mixed with 5 ml Million's reagent to obtain white precipitate. The precipitate was further warmed which turned to brick red precipitate which then dissolved giving red colour.

Ninhydrin test

To 3 ml test solution 3 drops of 5 % Ninhydrin solution was added and heated in boiling water bath for 10 min. It was then observed for purple to bluish colour.

Detection of Phytosterols

Libermann-Burchard's test

Few ml of the extract was taken and glacial acetic acid was added to it. To this solution 1-2 drops of conc. H₂SO₄ was added along the sides of the test tube. An array of colour changes shows the presence of phytosterols.

Detection of Glycosides

Legal's test

To the filtrate, 1 ml Pyridine and 1 ml sodium nitroprusside were added and then observed for pink to red colour.

Kellar- killiani test

To 2 ml extract, glacial acetic acid, one drop of 5 % FeCl₃ and conc. H₂SO₄ was added and observed for reddish brown colour at the junction of the two liquids and the upper layer appears bluish green.

Detection of Gums and Mucilages

To the filtrate, 10 ml of distilled water was added and to this 25 ml of absolute ethanol was added with constant stirring. White or cloudy ppt. indicates the presence of gums and mucilages [30].

Evaluation of antiurolithiatic activity: *In vitro* study

Collection of semi-permeable membrane from eggs shell

One side of the eggs was broken to squeeze out the content. Empty egg shells were washed with distilled water and kept in 2% (w/w) aqueous Hydrochloric acid solution for 24 hrs. After complete decalcification, the semi permeable membranes were collected and washed thoroughly with distilled water. Then neutralized with ammonia solution and finally rinsed with distilled water and stored in refrigerator at a pH of 7 to 7.4 in the moistened condition.

Preparation of solvents and reagents

- **Preparation of calcium oxalate solution:** 10 ml of 0.1% w/v aqueous solution of calcium oxalate.
- **Preparation of calcium phosphate solution:** 10 ml of 0.1% w/v aqueous solution of calcium phosphate.
- **Preparation of test solution:** 20 mg/ml aqueous extract of fresh fruits of *Elaeocarpus serratus* was taken as test drug.
- **Preparation of standard solution:** Cystone tablets were placed in absolute ethanol for removing colour coating and crushed into powder. 400 mg of powdered cystone was dissolved in 100 ml of distilled water and filtered and kept aside for further use.
- **Preparation of 0.02M KMnO₄ solution:** 0.32 gm of KMnO₄ was dissolved in 100ml of distilled water. It was boiled for 30 min. After cooling, excess of MnO₄ was removed by filtration.
- **Preparation of molybdate-sulphuric acid reagent:** Molybdate-sulphuric acid reagent was prepared by 5% w/v of sodium molybdate solution, 13 ml of conc. H₂SO₄ in 80 ml of distilled water. Finally, volume was adjusted to 100 ml with distilled water.
- **Preparation of reducing solution:** 1 gm of p-phenylene diamine was dissolved in 100 ml of 3 % w/v of sodium meta-bisulfite solution.
- **0.1 M Tris buffer:** Dissolve 8 gm of NaCl and 0.6 gm of Tris (hydroxymethyl) aminomethane in water and adjust the pH to 7.2 with 1M HCl and dilute to 1000 ml with water.

In vitro estimation of calcium oxalate

- Group I: 1 ml of calcium oxalate (1 mg/ml) + 1 ml of distilled water
- Group II: 1 ml of calcium oxalate (1 mg/ml) + 1 ml of Cystone solution (400 mg/ml)
- Group III: 1 ml of calcium oxalate (1mg/ml) + 1 ml of aqueous extract of *Elaeocarpus serratus* (20 mg/ml).

All the solutions of each group were packed individually in separate egg semi-permeable membrane and tied with thread. Then each packing was suspended in a conical flask containing 150 ml 0.1 M Tris buffer separately and covered with aluminium foil. All groups were kept in preheated (37°C) incubator for three days. The entire content of each group was transferred into test tube individually. 4 ml of 1N H₂SO₄ and 60-80 µl of 0.02M KMnO₄ were added and kept aside for 2 hours. Colour change from dark pink to colourless was observed after 2 hours. Change of colour intensity was measured against 620 nm in UV/Visible spectrophotometer. Concentration of undissolved calcium was determined.

In vitro estimation of calcium phosphate

- Group I: 1ml of calcium phosphate (1 mg/ml) + 1 ml of distilled water.
- Group II: 1ml of calcium phosphate (1 mg/ml) + 1 ml of Cystone solution (400 mg/ml).
- Group III: 1ml of calcium phosphate (1 mg/ml) + 1 ml of aqueous extract of *Elaeocarpus serratus* (20 mg/ml).

All the solutions of each group were packed individually in separate egg semi-permeable membrane and tied with thread. Then each packing was suspended in a conical flask containing 150 ml 0.1 M Tris buffer separately and covered with aluminium foil. All groups were kept in preheated (37°C) incubator for three days. The entire content of each group was transferred into test tube individually. 4 ml of 1N H₂SO₄ and 3 ml of molybdate sulphuric acid reagent, 1 ml of reducing solution were added and kept aside for 2 hours. Colour change from dark pink to colourless was observed after 2 hours. Change of colour intensity was measured against 620 nm in UV/Visible spectrophotometer. Concentration of undissolved calcium was determined by using the measured absorbance readings [31].

RESULTS

The organoleptic characters of *Elaeocarpus serratus* fruit powder is represented in Table no1. The ash values, which include total ash, water soluble ash and acid insoluble ash were performed and result is shown in Table no. 2. The moisture content and extractive values are represented in Table no. 3 and 4. Presence of carbohydrate, phytosterols, tannins were confirmed by phytochemical analysis (Table no. 5). Fig. 2 and Fig. 3 indicate the amount of calcium oxalate and calcium phosphate respectively (Table no. 6 and 7).

Table 1: Organoleptic characters of *Elaeocarpus serratus* fruit powder

Characters	Fruits
Colour	Green
Odour	Characteristic
Taste	Sour
Texture	Smooth
Shape	Oval

Table 2. Ash value of *Elaeocarpus serratus* fruit powder

Sl. No	Percentage (w/w) ash value
1	Total ash 2.73
2	water soluble ash 1.48
3	acid insoluble ash 1.04

Table 3. Extractive value of *Elaeocarpus serratus* fruit powder

S.No.	Solvent	Extractive Value (%)
1	Ethanol	34.84
2	Water	45.56

Table 4. Moisture content of *Elaeocarpus serratus* fruit powder

Weight of the powder taken (g)	% Loss in weight (w/w)	Mean value
1.5	0.103	
1.5	0.105	7.34
1.5	0.111	

Table 5. Phytochemical analysis of *Elaeocarpus serratus* fruit powder

Phytoconstituents	Petroleum ether extract	Aqueous extract
Carbohydrates and Reducing sugars	-	+
Alkaloids	-	-
Proteins and Amino acids	-	+
Tannins and Phenolics	-	+
Flavonoids	-	-
Phytosterols	-	+
Glycosides	-	-
Fixed oils	+	-
Gums and Mucilages	-	-
Saponins	-	-

Table 6. Dissolution of calcium oxalate

Group	Mean ± SD	Weight of Calcium reduced	Dissolution (%)
Group 1	0.160 ± 0.007	-	-
Group 2	0.075 ± 0.006	0.085	53.12
Group 3	0.113 ± 0.013	0.047	41.59

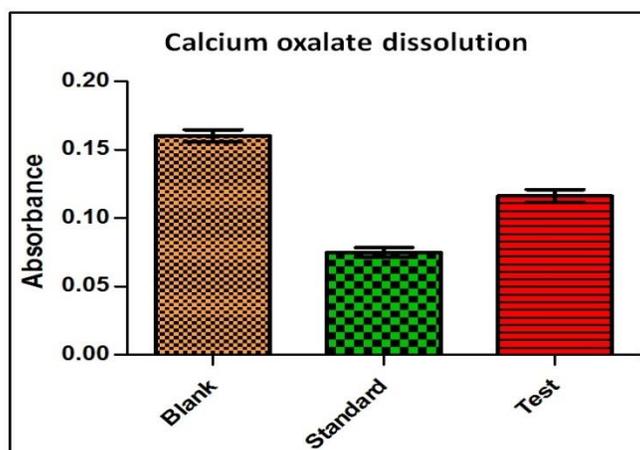


Fig. 2. Dissolution of calcium oxalate

Table 7. Dissolution of calcium phosphate

Group	Mean ± SD	Weight of Calcium reduced	Dissolution (%)
Group 1	0.131 ± 0.007	-	-
Group 2	0.058 ± 0.011	0.073	55.72
Group 3	0.089 ± 0.008	0.042	32.06

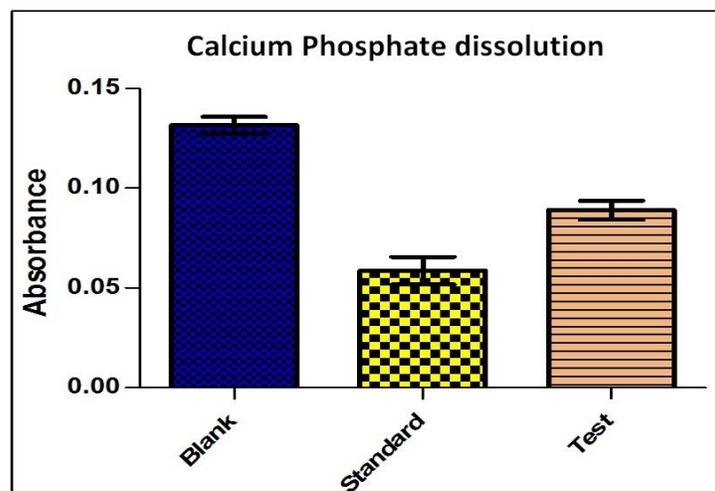


Fig. 3. Dissolution of calcium phosphate

DISCUSSION

The quality of a crude drug can be judged by determining its morphological (Table no. 1) characteristics and we found that the fruits of *Elaeocarpus serratus* is a drupe, oblong, ellipsoid or ovoid, bluntly pointed, smooth, dull, greenish yellow, pulp light to dark green, copious acid, edible and 2.5 cm long. It contains a much tubercle and one seeded stone. The total ash (Table no. 2) was found to be 2.73%, where as water soluble ash and acid insoluble ash were 1.48% and 1.04% respectively, which met the standard limit. Table no. 3 represents the percentage of water soluble extractives (45.56%) was found more than the ethanol soluble extractives (34.84%) which were up to the limit. The moisture content (Table no. 4) was found to be 7.34% w/w. All the experiments were performed in triplicate.

The Phytochemical analysis (Table no. 5) showing the presences of carbohydrate, proteins, amino acids, tannins, phenolics and phytosterols in aqueous extract and petroleum ether extract has given positive result for fixed oil.

Calcium oxalate and calcium phosphate in the urine form crystals on the inner surfaces of kidneys. This stage is called as initial mineral phase formation. Over the period of time crystals may combine to form a small, hard mass called as stones and stage is referred as crystal growth.

The extract of *Elaeocarpus serratus* has greater capability to dissolve calcium oxalate as the foremost

element for stone forming in the urinary tract. Lower percentage indicates more potency in dissolution of calcium oxalate crystals.

Table no. 6 represent a remarkable result as the highest percentage of calcium oxalate dissolution was observed in aqueous extract (53.12%) as compared to standard drug (41.59%). Table no. 7 also represent a good release of calcium phosphate by aqueous extract of *Elaeocarpus serratus* (55.72%) when compared with standard drug (32.06%). Figure no. 2 and Figure no. 3 represent that aqueous extract of *Elaeocarpus serratus* was found to be effective in the dissolution of calcium oxalate as well calcium phosphate when compared with standard drug (Cystone). This research work is providing a preliminary data on *Elaeocarpus serratus* fruits for removal of kidney stone, as the plant having lithotriptic property. Further research work is required to find out the compound responsible for this activity.

CONCLUSION

Traditionally *Elaeocarpus serratus* plays an important role in the treatment of various ailments. It is also used in Ayurvedic and Unani systems of medicine in India. In the present research work, the fruits of *Elaeocarpus serratus* were extracted and investigated for their physicochemical properties to determine their quality, safety and standardization for future utility. The present research work provides data which is very useful for the genuine

identification which may help in preventing adulteration. Further study is needed for the isolation, purification and characterization of medicinally active compounds from aqueous

extract of *Elaeocarpus serratus*. The pharmacological screening will be conducted to understand the mechanism of action.

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