ANTIUROLITHIATIC POTENTIAL OF *MUSA BALBISIANA* AND *MUSA ACUMINATA*

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ABSTRACT

The most common (about 80%) renal stones are calculi of calcium oxalate (CaOx) crystal. CaOx crystals, primary constituent of human renal stones, exist in the form of CaOxMonohaydrate (COM) and CaOxDihydrate (COD).Antiurolithiatic Potential of *Musa balbisiana* and *Musa acuminata* has been described. The aqueous extract of *Musa balbisiana* and *Musa acuminata* flower have inhibitory effect on CaOx crystallization thus may be beneficial in the treatment of Urolithiasis but there is a need of detailed investigation in elaborated preclinical experimentations and clinical trials to establish the use of plant as antiurolithiatic agent.

INTRODUCTION.

The formation of a kidney stone is a complicated problem that results from a succession of several physicochemical events including super saturation, nucleation, growth, aggregation and retention within the kidneys. Urinary stones affect 10–12 % of the population in industrialized countries

The common part of Recurrent stone formation in the medical care of patients with stone disease. Calcium-containing stones are most commonly occurring ones to an extent of 75–90% especially,Calcium oxalate monohydrateCalcium oxalates dehydrateBasic calcium phosphateMagnesium ammonium phosphate (Struvite) to an extent of 10–15%.Uric acid 3–10%.Cystine 0.5–1%. In most of the cases the commonly occurring stones are calcium oxalate or magnesium ammonium phosphate[4].

Many remedies have been employed during the ages to treat urinary stones. The use of plant products with claimed uses in the traditional systems of medicine assumes importance[5].

Herbal Medicines to Treat Urolithiasis

Human suffered from many types of kidney stones suggested by nutritionist , and treatment and prevention require wholesale changes in eating habits. A kidney stone, *calcium oxalate*, have low in fiber and high in refined carbohydrates, animal protein (including meat and dairy products), and alcohol. Protein can cause problems by prompting the body to lose more calcium in the urine, making it available for stone formation. Advised to eat food are vegetables, fruits, whole grains, and beans. A nutritional guidelines are recommended as vegetarian diet, that's no beef, poultry, or seafood.. Daily water consumption should be about four quarts, and specific foods, such as those high in oxalate should be cut from the diet. magnesium and Vitamin B6 Deficiencies occur may also lead to calcium oxalate stones. Several other supplements

may also be prescribed, including Vitamin K[6].The progenitor of modern edible bananas is *Musa acuminata*, along with *Musa balbisiana*. First cultivated by humans around 8000 years agoit is one of the earliest examples of domesticated plants.

Scientific Classification of *Musa acuminata*

Plantae: Kingdom
Zingiberales:Order
Musaceae:Family
*Musa:*Genus
acuminata :Species
Musa acuminate: Binomial name

The inflorescence grows horizontally or obliquely from the trunk(tightly packed layers of leaf sheaths). emerging from completely or partially buried cormsThe individual flowers are white to yellowish-white in color and are negatively geotropic. single inflorescence consist of male and female flowers. Female flowers develop into fruit, and the male flowers located at the tip most top-shaped bud in between leathery bracts.

Scientific Classification of *Musa balbisiana*

Plantae: Kingdom
Zingiberales:Order
Musaceae:Family
Musa: Genus

M. balbisiana:Species
Musa balbisiana: Binomial name

Musa balbisiana is a species of wild banana native to eastern and north in South Asiaand China. one of the modern cultivated bananas along with *Musa acuminata*.. Flowers grow in maroon and inflorescence colour. The present study aims to give data highlighting the two medicinal plants i.e., *Musa balbisiana* and *Musa acuminata* flowers accredited with Antiurolithiatic activity. This may help investigators to identify and develop appropriate lead compounds or plant products uses in the management of Urolithiasis.

MATERIALSAND METHODS

Sample Collection

The flower of*Musa balbisiana* and *Musa acuminata*was collected from Ponnappurrural areaatThanjavur district, Tamil Nadu.

Preparation of extract

The grinded flower material was subjected to soxhlet extraction separately and successively with Distilled waterat 60°C for 24hrs. These extract was concentrated to

dryness in evaporator under reduced pressure and controlled temperature (40-50^{o}C).The condensed extract was put in airtight container and stored in refrigerator.

Preliminary phytochemical analysis

The extract of *Musa balbisiana* and *Musa acuminata*was subjected to qualitative test for the identification of various plant constituents [33].

Test for Alkaloid

the extracts were treated with few drops of dilute hydrochloric acid and filtered. The filtrates were used for the following tests.

a. Mayers test

0.5ml of extract was treated with few drops of Mayer's reagent were added by the side of the test tube. A creamy white precipitate indicated the test as positive.

b. Dragendroff's test

0.5 ml of extract was treated with Dragendroff's reagent (potassium bismith iodide). Alkaloid indicates orange or orange red precipitate

c. Wagner's test

A few drops of Wager's reagent are mixed with 0.5 ml of extract gives an brown or reddish brown precipitate indicates the presence of alkaloid.

Test for Carbohydrate

5ml of distilled water mixed0.5 ml of extract and filtered. The filtrate was subjected to following tests to find the presence of carbohydrates.

a. Molish's Test

1 ml of Alpha napthol& Conc. H_2SO_4, treated with0.5ml of extract which gives a purple color.

b. Fehling's Test

0.5 ml of extract was treated with add equal qty of Fehling's sol A & B. After heating brick red precipitate was obtained.

Test for Phytosterols

0.5 ml of extract was dissolved in 5ml of chloroform separately then this chloroform solution was subjected to salkowaski and LibermannBurchard test for the detection of Phytosterols.

a. Libermann'sBurchard Test

0.5ml of extract was treated with few ml of chloroform, aceticacid and conc. H_2SO_4 which gives bluish green color.

b. Salkowaski Test

0.5 ml of extract was treated with chloroform was treated with Conc. H_2SO_4, gives red color.

c. Saponin Glycosides

0.5 ml of extract was treated with 80% H_2SO_4, gives deep yellow color indicates the presence of saponin glycosides.

Test for Saponins

a. Foam Test

Dilute 1ml of alcohol in 0.5 ml of extract separately with distilled water to 20ml and shake in a graduated cylinder for 15min. The formation of foam indicates the presence of Saponins.

Test for Tannins

0.5 ml of sample was treated with dye solution; formation of precipitate indicates the presence of Tannins.

0.5 ml of sample was treated with sodium acid phosphate and 2% Phenazone, formation of bulky precipitate often colored indicates the presence of Tannins.

Test for Flavonoids

0.5 ml of sample was allowed in a very few ml of ammonia. The mixture was determined below ultraviolet raditation and visual lights-formation of visible radiation color indicates the presence of flavonoids.

a. Shinoda's Test

0.5 ml of sample was treated with magnesium foil and conc. HCl given intense cherry red indicates presence of flavonoid. The orange red color indicates the presence of flavonols.

Test for Coumarin

0.5 milliliter of sample was treated with 100 percent binary compound, formation of yellow colour indicates the presence of Coumarin.

Test for flavones

0.5 milliliter of sample was treated with Na hydroxide; formation of yellow color indicates the presence of flavones.

Test for anthocyanin

0.5 milliliter of sample was treated with binary compound caustic soda indicates the presence of anthocyanin with the formation of blue violet color.

Quantitative analysis of secondary metabolites

Estimation of Alkaloid

The total alkaloid contents in the bark samples were measured using 1,10-phenanthroline method with slight modifications. 100mg bark powder was extracted in 10ml eightieth

grain alcohol.This was filtered through muslin cloth and centrifuged at 5000rpm for 10 min. Supernatant obtained was used for the further estimation total alkaloids. The reaction mixture contained 1ml plant extract, 1ml of 0.025M FeCl3 in 0.5M HCl and 1ml of 0.05M of 1, 10- phenanthroline in ethanol. The mixture was incubated for 30 minutes in hot water bath with maintained temperature of 70 ± 20oC. The absorbance of red colored advanced was measured at 510nm against chemical agent blank.

Estimation of Total Steroid

The sampleextract was determined by using a method described by (Singh *et al.*, 2004). 1 ml of sample extract was firstly mixed with 0.5ml of anisaldehyde, followed by addition of H2SO4. The mixture was then vortex for 10 mts and its absorbance was immediately measured at 600 nm by using spectrophotometer.

Estimation of Phenol

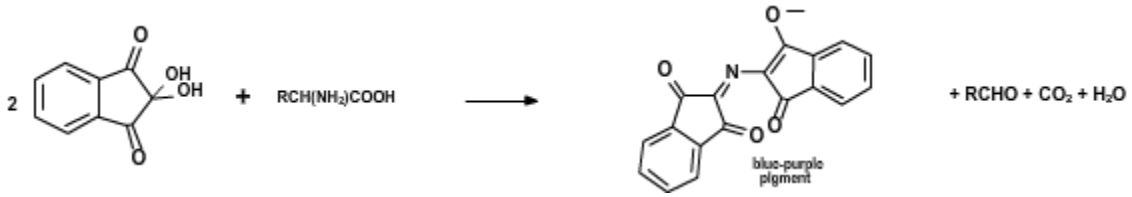
Folin-Ciocalteu (FC) reagent is used to determine total phenolic content . 0.75 ml of FC reagent (previously diluted 1000-fold with distilled water) is mixed with the plant extract (0.1 ml) and keep for 5 min at 22°C, then 0.06% Na2CO3 solution was added. After incubation at 22°C for 90 min, the absorbance was measured at 725 nm.

Estimation of amino acid by ninhydrin method

Principle

The ninhydrin reaction is one of the most reliable method for detection of amino acids in their microgram concentrations. Excess ninhydrin react with α-Amino acids to form a purple colored product, Ruhemann's purple. The amino acids proline and hydroxyproline (Imino acids) yield a yellow product. Under appropriate conditions, the color produced is proportional to the amino/imino acid concentration.

The overall reaction can be written as follows:



When Primary amines reacting with ninhydrin there will be no liberation of CO2. The absorbance of the Ruhemann's purple formed by the reaction is at 570nm is measured. For imino acids, the absorbance is done at 440nm. The principle behind the colorimetric estimation is given below:

Procedure:

Reagents required:

1. Standard amino acid stock solution.
2. 0.2M Acetate buffer (pH=5.5)
3. 4% w/v of Ninhydrin reagent [Preparation: weigh 4g of ninhydrin and dissolve in 100ml of acetone].
4. 50% ethanol
5. Distilled water.

2.4. dpph radical scavenging activity

DPPH Method Free radical scavenging potential of extracts was tested against a methanolic solution of DPPH (α, α- diphenyl - β -picrylhydrazyl) antioxidants react with DPPH and convert it to α, α-diphenyl- β - picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the inhibitor extract.The modification within the absorbance made at 517nm has been used as a live of inhibitor activity.

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Inhibitory activity of *musabalbisiana* and*musaacuminata*against caox crystals

The Inhibitory activity of *Musa balbisiana* and *Musa acuminata*were checked on Calcium oxalate crystals. The crude extract of both plant materials were taken at various concentrations and tested for its Antiurolithiatic activity.

0.1gms and 0.01gms of crystals were dissolved in two different concentrations of plant extracts. And also the Phytocompounds took in two different concentrations, such as 0.5ml and 1ml. The inhibitory activity was noted for about two days. The decrease in size of the crystals has been found only after 36hrs and then decrease in size took totally 48 hrs to dissolve completely.

Preparation of Artificial Urine: The artificial urine (AU) was prepared according to the method Burns and Finlayson and had the following composition:

1. Sodium chloride 105.5 mmol/l
2. Sodium phosphate 32.3 mmol/l
3. Sodium citrate 3.21 mmol/l
4. Magnesium sulfate 3.85 mmol/1
5. Sodium sulfate 16.95 mmol/1
6. Potassium chloride 63.7 mmol/1
7. Calcium chloride 4.5 mmol/1
8. Sodium oxalate 0.32 mmol/1
9. Ammonium hydroxide 17.9 mmol/1
10. Ammonium chloride 0.0028

Study without inhibitor: A volume of 1.0 ml of AU was transferred into the 0.5 ml of distilled water added to it and blank turbidity reading was taken. Then 0.5 ml of calciumoxalate was added, to the previous volume, and the measurement of the turbidity was immediately started for a period of 10 min. For each experiment, six replicates were done.

Study with inhibitor: Extract was resuspended in distilled water (0.25ml), a mixture of 1 ml of AU and 0.5 ml of plant extract solution is added. A blank turbidity reading was taken and then volume of 0.5 ml of calcium oxalatewas added and the measurement of the turbidity was immediately started for a period of 10 min. For each experiment, six replicates were done.

Calculation

$$\%\text{inhibition} = \{1-[Si / Sc]\} \text{ X } 100$$

Where; Si: slope of graph (Extract)
Sc: slope of graph (Control)

RESULTS AND DISCUSSION

The *In-vitro* study of Calcium oxalate crystallization and inhibition were analyzed in *Musa balbisiana* and *Musa acuminata*. The inhibitory activity was revealed the presence of Phytocompoundof those plants. This process is achieved by crystallization of calcium oxalate in synthetic urine. Antilithiatic activity is revealed by analyzing the changes in turbidity at 620nm by means of a spectrophotometer. The flower extracts of *Musa balbisiana* and *Musa acuminata* were analyzed for the presence of Phytochemicals. On the other hand, the calcium oxalate crystallization process was carried and the crystals were produced. The results are depicted as follows.

Quantitative Analysis of *Musa balbisiana* and *Musa acuminata*

This discussion help us to understand medicinally active constituents of the chosen plant. The phytochemical characters of the ten medicinal plants investigated are summarized in Tables 1.Alkaloids, steroid and phenol were present in selected samples.

Quantitative estimation of the percentage crudechemical constituents in these medicinal plants studied issummarized in Table 2. *Musa balbisiana*contained the highestpercentage crude yield of alkaloid and Phenol (34.4%, 36.8%), while *Musa acuminata*contained the high yield of steroid (11.9%).

The phytochemical study of the plant showed that theflowers of the selected plant were rich in alkaloids, steroid and phenol.There for they are exhibiting medicinal activity as well asexhibiting physiological activity.

Amino Acid Analysis of *Musa balbisiana* and *Musa acuminata*

In renal failure, it means nearly all of patients' kidney function has lost where a low-protein diet is needed, because excess protein can increase the workload on the kidneys. However, without enough protein, patients are more likely to feel fatigue, lack of energy and sleep problems, depression and anxiety. According to this analysis, we can know the root cause is that kidney failure patients can't get enough amino acid with the

protein limitation, so amino acid therapy, companying with low-protein diet, is good for these patients.

Amino acid such as Aspargine, Proline and valine wasfound to be high amount in *Musa acuminata,* whereas the leucine was highly present in *Musa balbisiana*when compare with *Musa acuminata.*(Table - 2)

In-vitro Antioxidant Activity of *Musa balbisiana* and *Musa acuminata*

There is increasing evidence that indigenous antioxidants may be useful inpreventing the deleterious consequences of oxidative stress and there is increasinginterest in the protective biochemical functions of natural antioxidants contained inmedicinal plants. The details of two flower from *Musa balbisiana* and *Musa acuminata*were selected in this study is described in table

Anti-oxidant activities of the samples were estimated by DPPH method. The aqueous extract of the sample shows higher activity in both extract. In aqueous extract of *Musa balbisiana*possess30% of activitywhere as the *Musa acuminata*showed 90% of activity respectively.(Table - 3)

*In-vitro*Anti Inflammatory Activity of *Musa balbisiana* and *Musa acuminata*

During inflammation the lysosomal enzymes released. And which produce a variety of disorders. The extra cellular activity of lysosomal enzymecorresponding to acute or chronic inflammation.Because of similarity of HRBC membrane to lysosomal membrane components the prevention of hypotonicity induced HRBC membrane lysis is used as a anti inflammatory measure of drugs.

The aqueous Extract of both *Musa balbisiana*and *Musa acuminata* shows significant anti inflammatory activity 71.7 % in "O" Positive blood group. The high level Anti inflammatory activity 75.3% was showed in aqueous extract *of Musa acuminata.* The anti inflammatory activity of the extracts were concentration dependent, with the increasing concentration the activity is also increased.(Table - 4).

In-vitro Antifungal Activity of *Musa balbisiana* and *Musa acuminata*

The antifungal activity of was assessed for *Musa balbisiana*and *Musa acuminata* againstselected pathogenic fungus namely, *Aspergillusniger, Cunninghamellabertholetiae, Penicilliumsp, Rhizobium mycorhizae*and *Candida albicans*.The inhibition of *Musa balbisiana*and *Musa acuminata*had good zone of inhibition against two fungal pathogens only such as, *Penicilliumsp*and *Rhizobium mycorhizae* the inhibition rate is 18mm for *Musa acuminata*extract and 29mm for *Musa balbisiana*respectively. There was observed a maximum inhibition rate against *Rhizobium mycorhizae*whereas the minimum inhibition rate was against *Penicilliumsp*for *Musa acuminata.* (Table - 5)

Table: 1 Shows quantitative analysis of Secondary metabolites

S. No	Name of the Test	*Musa balbisiana (%)*	*Musa acuminata (%)*
1	Alkaloid	34.4	28.7
2	Steroid	10.7	11.9
3	Phenol	36.8	33.9

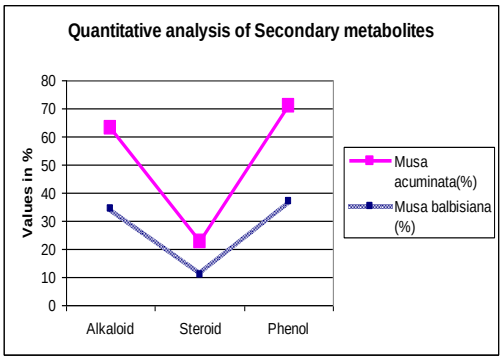


Table: 2Shows Amino acid analysis

S. No	Name of the Test	Value in mg	
		Musa balbisiana (%)	*Musa acuminata(%)*
1	Asparagine	9.03	18.15
2	Proline	8.42	10.3
3	Leucine	1.94	1.45
4	Valine	0.66	1.58

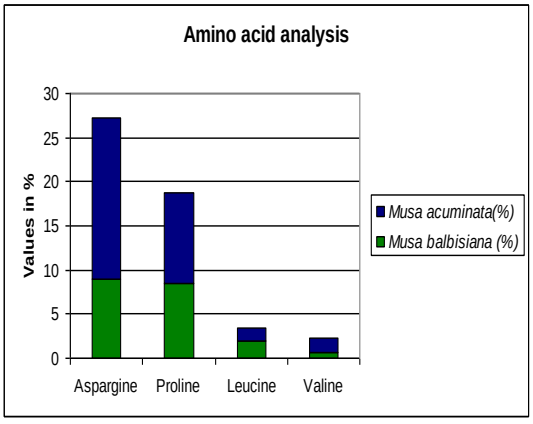


Table: 3 Anti Oxidant Activity DPPH Method

S. No	Name of the Test	Values in %	
		0.5	1.0
1	*Musa balbisiana*	11.5	30
2	*Musa acuminata*	41.75	90

Table: 4 *In-vitro* Anti Inflammatory Activity

S. No	Name of the Test	Values in %
1	*Musa balbisiana*	71.7
2	*Musa acuminata*	75.3

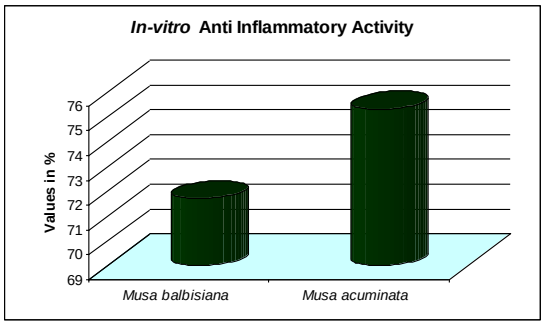
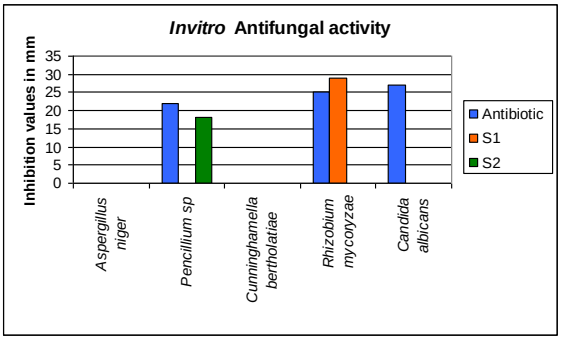


Table: 5*Invitro* Antifungal activity



Observation time	Inhibition Values in %	
	Musa balbisiana	*Musa acuminate*
Initial	30	32
30 mts	38	35
1 hr	52	50
1.30	60	59
2 hrs	73	68

CONCLUSION

Urolithiasis, formation of kidney stone-presence of one ormore calculi in any location within the urinary tract, is oneof the oldest and wide spread diseases known to man.The plant discussed here having wide range of application and uses. Further studies are going onthese plants in order to isolate, identify, characterize andelucidate the structure of the bioactive compounds. The anti-lithic activity of these flowers for the treatments of the kidney related problem as claimed by traditional healers is alsobeing investigated.

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