Research Article

Phytochemical Investigation and Evaluation of In-vitro Antilithiatic Activity of Muskmelon Fruit

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ABSTRACT

Muskmelon or *Cucumis melo L.* is one of the melon families that are widely consumed in many countries. The plant's ability to adapt to many climate condition makes it available in the market throughout the year. Phytochemicals is one of the important components in plant. In this study, the methanolic extract of muskmelon fruit were studied and tested for antilithiatic activity. The fruit have ability to brake or dissolute the stones present in the urinary tract.

Keywords: Muskmelon, urolithiasis, calcium oxalate, calcium phosphate, urinary stones.

INTRODUCTION

Urolithiasis is the word derived from Greek words *Ouron* (urine) and *lithos* (stone).Urolithiasis means formation of stone in the urinary system (calculi formed or located anywhere in the urinary system).[1] It comprises Nephrolithiasis, formation of stones in Kidney; Ureterolithaisis, formation of stones in the ureter, and cystolithiasis formation of stones in bladder.

Urinary tract stones

1.calcareous stones (calcium containing & radio-opaque) (75-90%)
a) Calcium oxalate (whewellite & weddellite)
b) Basic calcium phosphate
2. Non calcareous stones (non radio-opaque)
3. Struvite (Magnesium ammonium phosphate) (10-15%)
4. Uric acid (3-10%)
5. Cystine (0.5-1%)

About 80% of those with kidney stones are men. Men most commonly experience their first episode between20-30years of age, while for women the age at first presentation is somewhat later. Kidney stones typically leave the body in the urine stream, and a small stone comes in waves lasting 20 to 60 minutes. Other associated symptoms include: nausea, may pass without causing symptoms. If stones grow to sufficient size (usually at least 3 millimeters (0.1 in)) they can cause blockage of the ureter.
Typically vomiting, fever, blood in the urine, pus in the urine, and painful urination. Blockage of the ureter can cause decreased kidney function and dilation of the kidney. Most stones from due to a combination of genetics and environmental factors. Risk factors include being overweight, certain foods, some medications, and not drinking enough fluids. The diagnosis is usually based on symptoms, urine testing, and medical imaging. Blood tests may also be useful.[1]

The development of urinary stones is most commonly related to;

- Decreased urine volume
- Increased excretion of stone-forming components
- Inadequate urine drainage, which may lead to stasis

- Decrease in urinary citrate levels leading to deposition of calcium
- Deficiency of vitamins A or C-these conditions can also lead to the "Hypertraid":hyperparathyroidism, hypercalcaemia, and hyperuricosuria.

Ureteral obstruction causes postrenal Zotemia and hydronephrosis (distension and dilation of the renal pelvis and calyces), as well as spasm of the ureter. this leads to pain, most commonly felt in the flank (the area between the ribs and hip),lower abdomen, and groin(a condition called renal colic). Renal colic can be associated with nausea, vomiting, fever, blood in the urine, pus in the urine, and painful urination. Renal colic typically comes in waves lasting 20-60 minutes, beginning in the flank or lower back and often radiating to the groin or genitals. The diagnosis of kidney stones is made on the basis of information obtained from the history, physical examination, urinalysis, and radiographic studies. Ultrasound examination and blood tests may also aid in the diagnosis.

Among ruminants, uroliths more commonly cause problems in males than in females; the sigmoid flexure of the ruminant male urinary urinary tract is more likely to obstruct passage. Early-castrated males are at greater risk, because of lesser urethral diameter.[3]

Pelleted feeds may be conducive to formation of phosphate uroliths, because of increased urinary phosphorus ehcretion. This is attributable to lower saliva production where pelleted rations containing finely ground constituents are fed. with less blood phosphate patitioned into saliva more tends to be excreted in urine.(most saliva phosphate is fecally excreted).[8]

Oxalate uroliths can occur in ruminants, although such problems from oxalate ingestion may be relatively uncommon. Ruminant urolithiasis associated with oxalate ingestion has been reported. However, no renal tubular damage or visible deposition of calcium oxalate crystals in kidneys was found in yearling weather sheep fed diets containing soluble oxalate at 0.6% of dietary dry matter for about 100days.[10]

A person with recurrent kidney stones may be screened for such disorders. This is typically done with a 24 hours urine collection. The urine is analysed for features that promote stone formation.Calcium is one component of most common type of human kidney stones, calcium oxalate. Some studies suggest people who take calcium as a dietary supplement have a higger risk of developing kidney stones.

However, certain behaviors associated with frequent and binge drinking can lead to dehydration, which can in turn lead to the development of kidney stones. The American urological Association has projected that global warming will lead to an increased incidence of kidney stones in the United states by expanding the "kidney stone belt" of the southern United states.

People with lymphoproliferative/myeloproliferative disorders who were treated with chemotheraphy developed symptomatic stones 1.8%of of the time in one study.

PLANT REVIEW &PAST REPORT:

Melon is a very popular summer fruit in northern parts of India and annual climber growing to1.5m. Melon fruit is large, and can reach the size of football, although it is generally more flattened. several varities have been developed with varying pulp and skin colour and texture.

Common name: Melon, Muskmelon, Cantalopue, Honeydew, Sugar melon.
Synonyms: *Cucumis pubescens, Cucumis trigonus, Cucumis callosus*
Scientific classification:
Botanical name: *Cucumis melo*

Family: cucurbitaceae
Kingdom: Plantae
Order: cucurbitales
Genus: ***cucumis***

Muskmelon is a species of melon that has been developed into many cultivated varieties. These include smooth-skinned varieties such as Honeydew, Crenshaw and Casaba, and different netted cultivars (Cantaloupe, Persian melon, and Santa Clausor Christmas melon).The Armenian cucumber is also a variety of muskmelon, but its shape, taste, and culinary in this species approaches that found in wild cabbage, though morphological variation is not as extensive. It is a fruit of a type called Pepo.[11]
Muskmelon is native to Iran, Anatolia and Armenia, with secondary center including northwest India and Afghanistan. Muskmelons are monoecious plants. They do not cross with Watermelon, Cucumber, pumpkin or Squash, but variety in this species intercross frequently.[12] The genome of cucumis melo (L) was first sequenced in 2012.[13, 14]

NUTRITIOUS VALUE
Per 100gm serving, Cantaloupe melons provide 34 calories and are an excellent source of vitamin-A(68%) and vitamin-C(61%).Other nutrients are negligible level. Melons are 90% water 9%carbohydrates and 1%protein and fat.[15]
Cantaloupe is commonly known as muskmelon and kharbuja especially in India, however also famous as rock melon and sweet melon all over the World. It is a sweetest and amazing fruit having cooling effect on the body during whole summer season.

BENEFITS OF MUSKMELON
- Stimulate Digestive functioning and prevents their problems.
- Prevents Baby from Birth defects.
- Reduce risk of heart diseases..
- Protect from eye problems.
- Regulates Blood pressure.
- Prevents from hardening of Arteries.
- Prevents from Cancer.
- Prevents from Ageing.
- Regularizes Blood Sugar level.
- Promotes Hair growth.
- Prevents from Liver and Kidney problems.
- Prevents from Stone formation and Bone loss.
- Prevent from UTI.
- Protect from eye problems.

OBJECTIVE OF PRESENT STUDY
Fruits are health beneficial with variety of nutritious elements which are helpful for maintains of good health and are responsible for the treatment of variety of diseased conditions. Fruit extract is selected because its acceptability (sweet taste and pleasant flavor) while consumption but a crude drug might have bitter taste.
- To extract cucumis melo, cucurbitaceae fruit by using methol.
- To isolate the active principles present in the fruit by performing various chemical tests.
- To investigate the Antilithiatic activity of the methanolic extract of the fruit at different concentrations in-vitro.

MATERIALS AND METHODS
EXTRACTION PROCEDURE:
Fresh fruits of muskmelon were obtained local market, sliced in to small pieces and extracted with Methanol. The extract was concentrated and was used for Phytochemical investigation and for the determination of pharmacological activity.

Preparation of Semi permeable membrane

The semi permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin & yolk. Shell was removed chemically by placing the eggs in 2M HCL for overnight, 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. Then the egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while& rinsed it with distilled water. Stored in refrigerator at a PH of 7-7.4.

Preparation of standard solution

A polyherbal formulation such as Cystone was selected and tablets were placed in absolute methanol for removing colour coating and were crushed into powder form. The power was dispersed into 100ml of distilled water and filtered. Filterate was used as positive control.

ANTILITHIATIC ACTIVITY OF METHANOLIC EXTRACT OF CUCUMIS MELO

1) In-vitro Antilithiatic activity test by calcium oxalate dissolution method

Preparation of calcium oxalate by homogenous precipitation

1.47gm of calcium chloride dehydrate was dissolved in 100ml distilled water and1.34gm of sodium oxalate was dissolved in 100ml of 2N H2SO4. Both were mixed equally in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate was freed from traces of sulphuric acid by ammonia solution: washed with distilled water and dried at a temperature 60degree C for 2 hrs.

EXPERMENTAL PROTOCOL

control	1ml(1mg/ml) of calcium oxalate+1ml of water
test	1ml of calcium oxalate+1ml(10mg/ml)CMMC
test	1ml of calcium oxalate+1ml(20mg/ml)CMMC
test	1ml of calcium oxalate+1ml(30mg/ml)CMMC
test	1ml of calcium oxalate+1ml(40mg/ml)CMMC
standard	1ml of calcium oxalate+1ml(400mg/ml)cystone

CMMC: *cucumis melo* Methanolic extract

All the models were allowed to suspend in conical flasks containing 100ml of 0.1MTris buffer. All the flasks were subjected to incubated for three days. After three days the membranes were taken out of the flask and content of each membrane was collected in different test tubes. 2ml of 1N sulphuric acid was added to each test tube and titrated with 0.9494N KMNO4 till the colour disappears. 1ml of 0.9494N KMNO4 is equivalent to 0.1898mg of calcium.

The amount of undissolved calcium oxalate is subtracted from the total quality used in the experiment in the beginning; to know much quantity of calcium oxalate actually test substances could dissolve.

2) IN-VITRO ANTILITHIATIC ACTIVITY TEST BY CALCIUM PHOSPHATE DISSOLUTION METHOD

Preparation of calcium phosphate by homogenous precipitation

1.47gm of calcium chloride dehydrate was dissolved in 100ml distilled water and 1.42gm Of disodium hydrogen phosphate was dissolved in 100ml of 2Nsulphric acid. Both were Mixed equally in a beaker to precipitate out calcium phosphate with stirring. The resultant Calcium phosphate was freed from traces of sulphuric acid by ammonium solution; washed with distilled water and dried at temperature 60 degree C for 2hrs.

EXPERIMENTAL PROTOCOL

Control	1ml(1mg/ml)of calcium phosphate+1ml of water
Test	1ml of calcium phosphate+1ml(10mg/ml)CMMC
Test	1ml of calcium phosphate+1ml(20mg/ml)CMMC
Test	1ml of calcium phosphate+1ml(30mg/ml)CMMC
Test	1ml of calcium phosphate+1ml(40mg/ml)CMMC
standard	1ml of calcium phosphate+1ml(400mg/ml)cystone

CMMC: cucumis melo Methanolic extract

All the models were allowed to suspended in conical flasks containing 100ml of 0.1MTris buffer. All the flasks were subjected for 3 days. After 3 days the membranes were test out of the flask and content of each membrane was collected in different test tubes. 4ml of 1N sulphuric acid 3ml of molybdate-sulphuric acid reagents. 1ml of reducing solution were added and kept a side for 2hrs. Colour changefrom dark pink to colour less was observed after 2hrs.Change in colour intensity was measured against 620nm spectrophotometrically. Concentration of undissolved calcium was determined from standard calibration curve of calcium phosphate by using measured absorbance readings.

$$\%\text{inhibition} = \{1-[si/sc]\} \times 100$$

Where; **si**: slope of graph in the presence of inhibitor (plant extract),
Sc: slope of gragh without inhibitor (control)

RESULTS AND DISCUSSTION

Phytochemical Investigation

Methanolic extract of fruit has shown the presence of Carbohydrates and Fats, Proteins and Vitamins.

PHYTOCHEMICAL INVESTIGATION

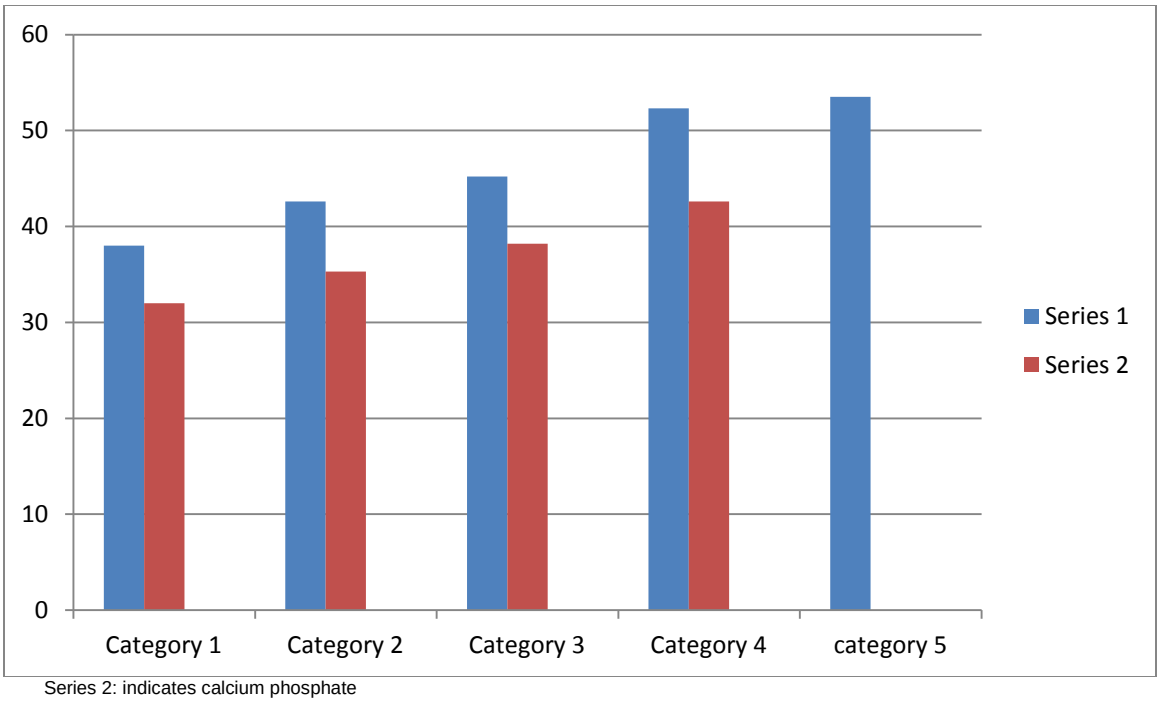
Chemical test for the Methanolic extract of *Cucumis melo*

NAME OF TEST	METHANOLIC EXTRACT
Triterpenes: A) Salkowski test **B)** Liebermann-Buchard's test	+ +
Carbohydrates: A)Molisch's test: B) Fehilings test: C) Benedicts test: D) Barfoeds test:	+ + + +

In-vitro activity Antilithiatic activity test by calcium oxalate dissolution method

Effect of Methanolic extract was statistically equal to the effect of standard drug being used for dissolving the existing renal stone.Methanol extract of 4 concentrations were taken for experimental purpose (10mg/ml, 20mg/ml, 30mg/ml, 40mg/ml). Dissolution of crystals were less for Methanolic extract of 10mg/ml and 20mg/ml concentrations. Whereas the extract of 30mg/ml, and 40mg/ml has shown almost similar of dissolution of crystals as that of standard cystone of 400mg/ml.

Treatment Groups	Calcium oxalate Absorbance At620nm	Calcium oxalate %Dissolution	calcium phosphate Absorbance At620nm	calcium phosphate %Dissolution
Control	1.562	0.0	1.562	0.0
methanolic extract10mg/ml	0.432	38±0.02	32.0±0.02	32.0
methanolic extract20mg/ml	0.536	42.6±0.02	35.3±0.02	35.3
methanolic extract30mg/ml	0.578	45.2±0.02	38.2±0.02	38.2
methanolic extract40mg/ml	0.623	52.3±0.03	42.6±0.02	42.6
Standard	0.650	58.4±0,02	58.4±0.02	58.4



Series 2: indicates calcium phosphate
Series 1; indicates calcium oxalate
%dissolution of calcium oxalate & calcium phosphate

CONCLUSION

The existing fact came out of the study is that the Methanolic extract of plant showed statistically equal potential as compared to standard drug Cystone in dissolving the artificial stone crystals even in the crude form. Also the results came out of regarding the dissolution experiment have great importance as Methanolic extract in crud form got second position in effect after the standard drug Cystone. Crystal dissolution by the Methanolic extract is of great medical science interest. As it is having sweet taste and rich source of fiber and nutritious elements, due to reported activities better to consume it in all seasons.

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