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In-vitro Anti-urolithiatic Activity of carica papaya roots

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ABSTRACT

The fruit of Carica papaya Linn (Caricaceae) used in traditional medicine for the treatment of urinary stones. The present study was undertaken to evaluate theantiurolithatic effects of the aqueous and alcoholic extracts of the fruit of C. Papaya on ethylene glycol (EG) induced urolithiatic rats. EG administration resultedin hyperoxaluria as well as increased renal excretion of calcium and phosphate. Treatment with aqueous and alcoholic extracts of C. papaya fruit significantlyreduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in thekidneys of calculogenic rats was also significantly lowered by curative and preventive treatment using aqueous and alcoholic extracts of the fruits of C. papaya. The results indicate that the fruit of C. papaya is endowed with antiurolithiatic activity and scientifically valid the traditional use of the fruit of C. Papaya in the treatment of urinary calculi.

Key words: Hyperoxaluria, Kidney stone, Microcrystal, Renal damage

INTRODUCTION

The Ayurveda system of Indian medicine and science of life deals with health problems of human being. The three authors named as charaka, susruta and vagbhata support the scientific methods of study to promote the perception of Ayurveda towards humanity.

In Ayurveda, urinary calculi are known as Mutrashmari According to Sushrutacharya, father of surgery urolithiasis is dreadful as it considered is “Astamahagadhas” which means in “incurable disease”.

He also explained that formation of stone is complex physiochemical process which involves a series of events as urinary saturation- superstation-nucleation-crustal growth -crystal retention-stone formation.

Urolithiasis

Urolithiasis is the formation of stone in urinary tract. These stone belong to the group of biominerals, that is different organic and inorganic substances with a crystallin or amorphous structure.

- It is a precipitation of insoluble and less soluble salt such as oxylate and phosphate which causes obstruction of urethra which results in renal colic, haematuria, pyuria, Dysuria and oliguria.
- It is tangle process that is outcome of imbalance between promoters and inhibitors in kidneys.

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- The oldest bladder stone is reported as "Elamararah" in upper Egypt, dated about 4800 B.C., which shows that human of ancient time was affected with this disease Ashmari similarly as now a days.

It is the one of the most common disease found in the world which has condition of reoccurrence in nature in malignancy of removal of stone by surgical method in large no of cases.

The rate of occurrence in three times higher in men in comparison to women because of enhancing capacity oestrogen in stone formation.

Table 1.1 Types of stone: Stones are differing due to its mineral composition:

S. No.	Name of Stone	Occurrence %	Combination
1.	Calcium oxylate	70-80	Calcium, oxylate
2.	Calcium phosphate	5-10	Calcium, phosphate
3.	Uric acid	5-10	Uric acid
4.	Struvite	10-20	Calcium, ammonia, phosphate
5.	Cystine	Less than1	Cystine

1. **Calcium oxalate:**
   - It is also known as mulberry stone. It is covered with sharp projections which cause haematuria (kidney bleeding).
   - It is radio opaque and looks like dumble shape in microscope. If it is monohydrate & looks like envelop if it is dihydrate.

2. **Calcium phosphate:**
   - It is also called Brushite and it appears like needle when observe under microscope.
   - It enlarges rapidly in alkaline urine & and take shape like sepals of flower.
   - It is dirty white in colour, radio opaque & 1 cm in diameter.
   - Formation of calcium phosphate stone in related with hyperparathyroidism & renal tubular acidosis.

3. **Uric acid stones:**

   - The uric acid stone may be hard or may be smooth
   - Uric acid stones may produce due to certain abnormalities such as obesity.
   - Disorders of acid/base metabolism due to which excessive acid is present in urine, also results in formation of uric acid crystals.

- These stone are round in shape & yellow or reddish -brown in colour.
- They are radio translucent or transparent unless mixed with calcium crystals or struvite.
- It looks like irregular plates or cluster under microscope.

4. **Struvite stones:** Struvite stone is also known as "infection stone", because of the presence of infection by urea splitting bacteria such as proteus mirabilis, proteus vulgaris, mprganella morganii, klebsiclla serratia, Enterobacter and pseudomonas species.

- These organisms metabolize urea into ammonia & carbon dioxide which alkalinizes the urine and provide the favourable condition for the formation of struvite stones.
- Occurrence of struvite stone is more common in people having metabolic disorders such as idiopathic hypercalciuria, hyperparathyroidism & gout.
- Radiographs of struvides stones are shown as large, gnarled and laminated.

5. **Cystine stone:** It an autosomal recessive disorder and commonly occurs in young girls which is due to cystinuria (excess of cystine in the urea)

Cystinuria is gentic disorder of transport of amino acid in excess amount of cystine which is lest soluble in all amino acids. Thus, it precipitates out and form stones in urinary tract.

- They are pink or yellow in colour with shiny crystalline & radio opaque.
- They appear like hexagonal or benzene ring under microscope.

- The existing scenario of urolithiasis is nearly about 4-15% of human population are suffering from urinary stone problem all over the world. In India 5-7 million people are suffering from this disease where its rate of occurrence in 3 times higher in men in comparison to women. The problem of urinary stone formation is considered as a medical challenge because of its various causes & high rate of reoccurrence.

1.2 Research Objective:

1. **Aim:** A quasi experimental study on the effects of *carica papaya* root extract to inhibit stone formation and dissolution of already existing stone in renal system using in-vitro method in the laboratory.

2. **Objective:**
   1. To examine possible medicinal action of herbal remedy on kidney stone inhibition.
   2. To evaluate the antiurolethetic effect of aqueous, alcoholic and hydroalcoholic extract of the roots of *carica papaya* on synthetically prepared calcium oxalate crystal.
   3. To compare the dissolution of calcium oxalate crystal at different doses of extract of *carica papaya* roots.

1.3 Plan of Work:

- Literature survey
- Collection of plant material
- Authentication of Plant
- Preparation of Various extracts (Aqueous, Ethanolic and Hydro-alcoholic)
- Evaluation of antiurolithiatic activity using *in-vitro* calcium oxalate dissolution method.
- Evaluation of antiurolithiatic activity of three different extracts from *carica papaya* roots.
- Preparation of thesis.

2.2. Herbal Drug possessing antiurolithiatic activity:

S. N.	Vernacular Name	Botanical name	Part use	Animal model/ study model	Reference
1.	Papaya	*Carica papaya*	Seed, Fruit	Male Wister rat *In-vivo* assay	Nayeem khatib *et al*,2010
2.	Shatawari	*Asparagus racemosus*	Root	*In-vitro* method	Tiwari A. *et al*,2012 and Mikawlrawng K. et al,2014
3.	Patharkuchi	*Bergenia ligulate*	Rhizome	*In-vitro* turbidity assay, *In-vivo* animal	Yadav R.D *et al*,2011 and Ram J.et al,2010
4.	Tejpata	*Cinnamomum tamala*	Leaves	*In-vitro* assay	Mikawlrawng K. *et al*,2014
5.	Nimbu	*Citrus limon*	Fruits	*In-vitro,In-vivo* animal assay	Ram J.*et al*,2010 and Touhami M *et al*,2007
6.	Copaiba	*Copaifera langsdorffii*	Leave	*In-vitro,In-vivo* animal assay	Oliveira RB *et al*,2013
7.	Varuna	*Crataeva nurvala*	Bark	*In-vivo* animal assay	Tiwari A. *et al*,2012, Mikawlrawng K. *et al*,2014 and Yadav R.D *et al*,2011
8.	Turmeric	*Curcuma longa*	Rhizome	*In-vivo* animal assay	Tiwari A. *et al*,2012 and laksmi

					N.V.
9.	Kulthi	*Dolichos biflorus*	Seed	*In-vitro,,In-vivo* animal assay	Tiwari A. *et al*,2012, Yadav R.D *et al*,2011
10.	Kurchi	*Holarrhena antidysentrica*	Seed	*In-vitro, In-vivo* animal assay	Khan A *et al*,2012
11.	Senjana	*Moringa oleifera*	Root,bark and flower	*In-vivo* animal assay	Tiwari A. *et al*,2012, Yadav R.D *et al*,2011, laksmi N.V., Muneer KC and Thenmozhi C.
12.	Jangali amli	*Phyllanthus niruri*	Leave	*In-vitro, In-vivo* animal assay	Yadav R.D *et al*,2011, Ram J.*et al*,2010
13.	Passhana bhed	*Rotula aquatica*	Leavs, stem and roots	*In-vitro, In-vivo* animal assay	Ram J.*et al*,2010 and Muneer KC
14.	Imli	*Tamarindus indica*	Leaves	*In-vivo* animal assay	Mikawlrawng K. *et al*,2014
15.	Arjuna	*Terminalia arjuna*	Bark	*In-vitro* assay	Mittal A. *et al*,2015 and 2016
16.	Gokhru	*Tribulus terrestris*	Roots, whole plant, fruits and leaves	*In-vivo* animal assay	Yadav R.D *et al*,2011, laksmi N.V. and Thenmozhi C. and bouabdelli F. *et al*,2012
17.	Cucumber	*Cucumis sativas linn.*	Leaves	*In-vitro* assay	Choubey Ankur *et al*, 2010
18.	Lantana	*Lantana camara linn.*	Leaves	*In-vitro* assay	Mayee *et al*, 2011
19.	Black night shade	*Solanum indicun linn*	Roots	*In-vitro, In-vivo* animal assay	Prachi *et al*, 2009

2.3 Review of literature on *Carica papaya* plant:

S. No.	**Vernacular Name**	**Botanical Name**	**Part used**	**Activity**	**Reference**
1	Papaya	*Carica Papaya Linn.*	Leaves	Anti-microbial activity	Tiwari B Drij *Et al*, 2014

2	Papaya	*Carica Papaya Linn.*	Seeds	Anti-ulcerogenic activity	Oloyede. E. O. Hussein *et al*, 2015
3	Papaya	*Carica Papaya Linn.*	Seeds	Anti-fertility activity	Pudoh *et al*, 1999
4	Papaya	*Carica Papaya Linn.*	Latex	Anthelmentic activity	Kanthal Kantalakshmi 1 *et al*, 2012
5	Papaya	*Carica Papaya Linn.*	Roots	Anthelmentic activity	G. Araviand et al, 2014
6	Papaya	*Carica Papaya Linn.*	Roots	Dysspepsia	ES Carlos *et al*, 2008
7	Papaya	*Carica Papaya Linn.*	Roots	Urethritis	JH coughari *et al*, 2017

2.4Plant Profile:

a) **Plant name:** Papaya
b) **Biological source:** It consist of different parts of *carica papaya* plant including leaves, seeds, latex, fruits and roots.

Fig.2.1 Plant of *Carica Papaya*

Fig. 2.2 Root of *carica papaya*

Synonyms:

a) Indian synonyms of *Carica papaya* Linn**.**

S.N.	Language	Region	Names
1.	Hindi	Haryana, Delhi	Papaya, Papita

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2.	Bengali	West Bengal	Papaya, pepe, papita
3.	Malayalam	Kerala	Omakai
4.	Panjabi	Panjab	Papita
5.	Marathi	Maharashtra	Papai
6.	Tamil	Tamil Nadu	Pappali
7.	Gujrati	Gujrat	Papaya
8.	Kannael	Karnataka	Pharangi

b) **Morphology:**

- **Life stage and characteristic**
  Plant Division: Angiosperms (Flowering Seed Plants) (Dicotyledon)
  Plant Growth Form: Shrub (Herbaceous)
  Mode of Nutrition: Autotrophic
  Plant Shape: Umbrella
  Maximum Height :2 m to 10 m

- **Other Features**
  Propagation Method: Seed
  Light Preference: Full Sun
  Water Preference: Moderate Water
  Plant Growth Rate: Fast

Flower & Plant Sexuality:Unisexual & Bisexual Flowers (Sub-dioecious)

c) **Geographical distribution:**

- The plant is native to tropical America and was introduced to India in 16th century.
- Successful commercial production today is primarily in Hawaii, tropical Africa, India. The Philippines, Ceylon, Malaysia and Australia apart from the widespread but smaller scale production in South America and Latin America.
- In India, papaya is cultivated in Maharashtra, Bengal, Bihar, Haryana, Panjab, Delhi, Andhra Pradesh and Uttar Pradesh.

d) **Cultivation of carica papaya:**

1. **Climate and soil condition**

- Very sensitive to forest.
- Optimum temperature is 25-30°C and minimum temperature is 16°C.
- Soil is well drained or sandy loam soil with adequate organic matter is most important
- Climate is dry at the time of ripening is good for fruit quality.

2. **Growing season:**

- In northern part of Taiwan, seeds are sown form march to May and transplanted from May to July.
- In central & south areas seeds are sown almost all years round, but optimum season is February to march (Spring) or from September to November (Autumn).

3. **Sowing method:** It may be sown directly, but normally, it is better to be seeded to raise seedling and transplanted.

4. **Transplanting:**

a. **Transplanting stage:** When the seedling are 10-15cm long, it should be transplanted, but 30-40 cm large container, all right if it is grown in a large container.

b. **Spacing:** a 40-60cm high bed is required if the soil is not well drained. Normally distance between rows is about 2-2.5 and 2m between plants.

5. **Fertilization:** The plant needs continuous fertilization, as fruiting is continuous upon maturity.

a. Basal fertilization: Apply 10tons of fermented compost per hectare (or 1kg/sq.) before planting or when forming beds. The same dose should when forming beds. The same dose should be repeated every year for adult plants.

b. Side dressing: NPT at rate of 4:85 monthly, bimonthly or seasonally.

c. Others:
   - Apply 0.25-0.5kg borax per100 plants right before dry season.
   - For young trees, apply compound fertilizers in the trench (10cm deep &15cm wide).

6. **Harvest:** in general papaya takes six months to flower and anther five for harvesting, but it may vary according the climate conditions and management. For shipping to the distant markets, the fruits should be harvested when the apical starts turning yellow and the latex is no longer milky. During the cold months the fruits can be left on the tree to develop deeper colour and obtain better flavour.

e) **Ethnomedical use:**

S.N.	Part	Properties
1	Leaves	In some parts of Asia, the young leaves of the carica papaya are steaned & eaten like spinach to facilate digestion. (G. Aravind et al, 2013) Papaya leaves are made into tea as treatment for malaria. (Yogi raj Vijay *et al*, 2014) In India leaves are used for colic, fever abortion

		and asthma. In Australia papaya leaves are used traditionally in treatment like jaundice, malaria, dengue, Immunomodulatory. (KM Basalingappa *et al*, 2018)
2	Flower	It is used in the treatment of Jaundice. (KM Basalingappa *et al*, 2018)
3	Fruits	The milky juice from the green mature fruits is tapped, while still in the tree contain an enzyme papain, people used in preparation of different remedies of indigestion. (KM Basalingappa *et al*, 2018)
4	Peel	Peels are used in many home remedies such as sunscreen and soothing slave, fight dandruff. Used as muscle relaxant with the addition of vinegar in its oil. (Yogi raj Vijay *et al*, 2014)
5	Roots	In some Countries of Asia it is used to ease urinary troubles. (Yogi raj Vijay *et al*, 2014) Roots is chewed and swallowed for treatment of cough, bronchitis and other respiratory disease. (KB bergenin *et al*, 2016) They used in traditional medicinal as a great source of drug of birth control in males. (CO nwaehujora et al, 2014)
6	Seeds	In some Countries of Asia, it is used to ease urinary troubles. (Yogi raj Vijay et al, 2014) Roots is chewed and swallowed for treatment of cough, bronchitis and other respiratory disease. (KB bergenin et al, 2016) They used in traditional medicinal as a great source of drug of birth control in males. (CO nwaehujora et al, 2014) It also helps in treating infections, wounds, abdominal pain. (KM Basalingappa et al, 2018)

f) **Chemical constituents of various parts of carica papaya:** (Yogi raj Vijay et al, 2014, G. Aravind et al, 2013)

S. N.	Part	Chemical Constituents
1.	Fruit	Protein, fat, Fibres, carbohydrate, minerals, calcium, phosphate, iron, thiamine, riboflavin, ascorbic acid, niacin,

		coronene, amino acid, Citric acid, molic acid, volatile compounds like linalool, benzyl isothiocyanate, cis and trans 2,6-dimethyl-3,6 enpony- 7 octen-2-ol, alkaloids, β-D glucoside derivatives and four isomeric malonated benzyl β-D glucose.
2.	Juice	n-butyric, n-henaoic and n-octanoic acid, lipids like myristic, palmitic, stearic, linolenic and oleic acids.
3.	Seed	Fatty acids, crude proteins, crude fibres, papaya oil, carpine, benzyl isothiocyanates, benzyl glucoside, gluctnopacolin, benzyl-thiourea, hentriacentone, β-sitosterol, caricin and an enzyme myrosin.
4.	Root	Arposide and an enzyme myrosin.
5.	Leaves	Carpain, pseudocarpain, dehydrocarpain I and II, Cholinic caricine, vitamin C and E.
6.	Bark	β-sitosterol, glucose, fructose, sucrose, galactose and xylitol
7.	Latex	Proteolytic enzymes, papain, chymopapain, glutamine, chymopapain A, B and C, peptidase A,B and lysozyme.

2.5 Pharmacological activity reported: (Yogi raj Vijay *et al*, 2014, Gunde. C Mahendra *et al*, 2015)

1) **Anti-inflammatory activity:** The anti-inflammatory property of plant cysteine proteinases was already noted in literature. In a clinical study, the histological severity of inflammatory bowel disease was determined for treatment of chronic inflammatory and related diseases papain has found to be safe and efficacious.

2) **Anthelmintic activity:** A wide range of plants and plant extracts has been used traditionally for the treatment of helminths infections including papaya, which is rich in proteolytic enzymes known to digest nematode cuticles, have low toxicity and have been used in traditional medicine against gastrointestinal nematodes for decades.

3) **Antifungal activity:** The latex of papaya and fluconazole has synergistic action on the inhibition of Candida albicans growth. This synergistic effect results in partial cell wall degradation due to lack of polysaccharides constituents in the outermost layers of fungal cell wall and release of cell debris into the cell culture.

4) **Antibacterial activity:** The seeds of *Carica papaya* were found to possess bacteriostatic activity against several enteropathogens such as *bacillus subtilis,*

enterobacter cloacae, escherichia coli, salmonella typhi, staphylococcus, proteas vulgaris, pseudomonas aeruginosa and *klebsiella pneumonia.*

5) **Anticancer activity** Initially pharmaceutical preparations containing various proteolytic enzymes (papain) have been used as adjuvant in the treatment of malignant diseases, despite lack of knowledge of their mode of action. Experiments indicate that the effects after oral administration of poly enzymes preparations are related to the induction of cytokines production by human peripheral blood mononuclear cells.

6) **Immunomodulatory activity:** Papain induces human eosinophils to degranulate and to produce superoxide anion. The E-64 inhibitors abolished the activation by papain suggesting that the protease activity is required to trigger eosinophil response. It is likely that this action in eosinophils is mediated by protein G linked receptor.

7) **Anti- sickling activity:** Sickle cell disease (SCD) results from a mutation in haemoglobin inside the red blood cells, where a glutamic acid at 6th position is replaced by valine. Recent studies showed that unripe papaya fruit extract has anti-sickling activity.

8) **Hypoglycemic and hypolipidemic activity** Study show that oral treatment with 0.1 mg/kg/day of glibenclamide and 100-400 mg/kg/day of aqueous seed extract of Carica papaya induced significant, steady and progressive hypo-glycemic and hypo-lipidemic effect.

3.1 *In-vitro* anti-urolithiatic activity by calcium oxalate dissolution method:

3.1.1 Preparation of Calcium oxalate crystal:

1. **Solution 1:** Solution of calcium chloride was prepared by taking 10gm of calcium chloride dissolved in 10ml of distilled water.
2. **Solution 2:** 10gm of sodium oxalate in 10ml of 2N H_2SO_4.
3. Then mix the both solution in equivalent quantity and add 2ml of tris buffer (pH 7.4).
4. Then the solution was placed for incubation at 37ºC for 12 hours and after that the solution was centrifuged for 10min and decant supernatant liquid.
5. These Calcium oxalate crystals were washed with ammonia solution and distilled water to make free from trace of sulphuric acid and dried at 60°C thus, result in formation of calcium oxalate crystal.
6. The prepared calcium oxalate crystals formed were checked using the compound microscope under 45x magnification. The crystals formed resembles prism shape.

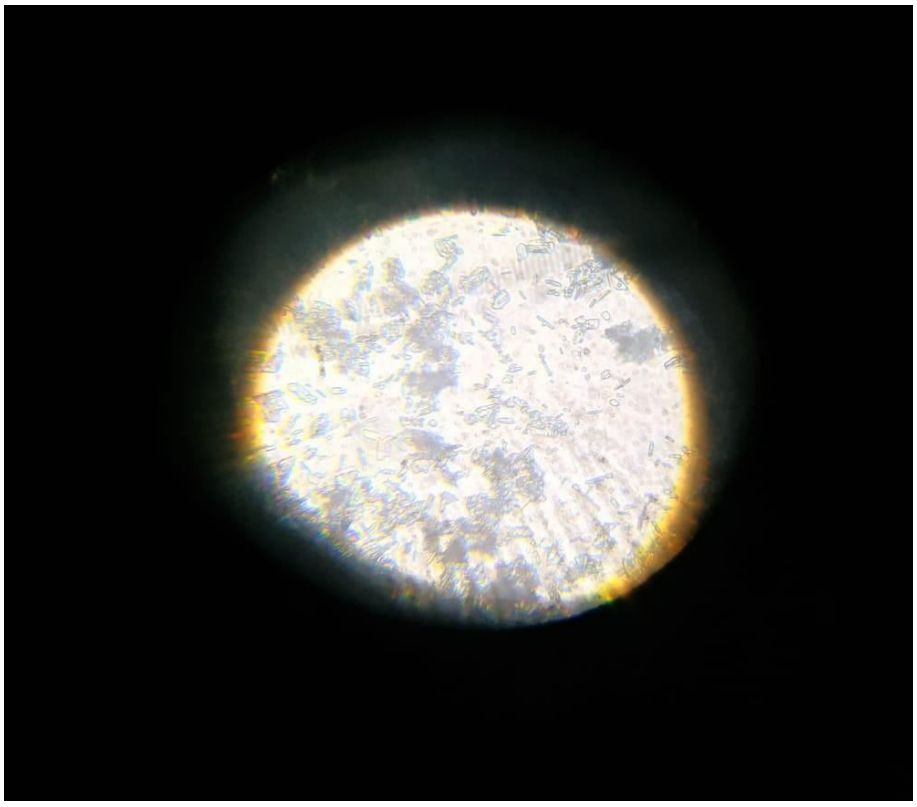

Fig. 3.1 Microscopic view of prepared Calcium oxalate crystal

3.1.2 Procedure for *In-vitro* anti-urolithiatic activity:

1. 5ml of each three types of dilution that were (10mg/ml, 40mg/ml, 80mg/ml) of three different extracts (Aqueous, Alcohol, Hydroalcoholic) of carica papaya roots were introduced into individual petri-dishes having calcium oxalate crystals.
2. All of the treating agents were incubated at 37ºC for three days.

On the 4th day all the petri-dishes were taken and dissolution of crystal was checked under the compound microscope at 45x magnification.

MATERIAL AND METHODS

3.2 List of glassware used in experiment:

S. No.	Name	Specification (ml)
1.	Beaker	50,250,500
2.	Measuring cylinder	25,50,100
3.	Test tube	20
4.	Glass road	-
5.	Petri dishes	-

6.	Pipettes	5,10
7.	Funnel	-
8.	Evaporating dish	-
9.	Spatula	-
10.	Silica crucible	-
11.	Droppers	-
12.	Iodine flask	250

3.3 List of Equipment's used in experiment:

S. No.	Equipment's Name
1.	Magnetic Stirrer
2.	Heating Mental
3.	Hot Air oven
4.	Water Bath

3.4 List of Chemical's used in experiment:

S. No.	Name of Chemicals
1.	Sodium oxalate
2.	Calcium Chloride
3.	Sulphuric acid
4.	Ammonia Solution
5.	Ethanol
6.	Chloroform
7.	Sodium bicarbonate
8.	Distilled water

3.5 Collection of plant Material: The roots of carica papaya was collected from the local village of the Dehradun and collected plant material subjected for preparation of herbarium file and sent to for the identification and authentication.

3.5.1 Herbarium of *Carica Papaya*:

- **Kingdom:** Plantae
- **Division:** Spermatophyte
- **Class:** Magnoliopsida
- **Order:** Brassicales

- **Genus:** Carica
- **Family:** Caricaceae
- **Locality:** Bhauwala, Dehradun
- **Habit:** Evergreen tree
- **Remark:** Tree
- **Collected by:** Prerna Upadhyaya
- **Identified by:** Dr. Praveen Kumar

The herbarium of the plant specimen has been deposited to Systemic Botany Discipline, Forest Research Institute, Dehradun and the voucher specimen No. 129/Dis./2019/Syst.Bot./Rev.Gen./4-5.

3.6 Plant Material: The roots of plant carica papaya was collected from Bhauwala, Dehradun, Uttarakhand. Roots sample were collected in month of march to April 2019. Then the roots were dried.

3.7 Preparation of Extracts: Carica papaya roots were cut into slices and dried in shade for 5 days. Then dried roots of the plant were grinded into uniform powder with the help of electric blender.

After that the powder drug was used to prepare three extracts in three different solvents by Cold maceration method.

3.7.1 Aqueous extract:

- 10gm of carica papaya roots was taken in to a iodine flask along with 100ml of distilled water.
- 5ml of chloroform was added into iodine flask for avoiding microbial contamination or preservation and Cold maceration method was employed.

3.7.2 Alcoholic Extract:

- 10gm of powdered roots of carica papaya was taken into Iodine flask and 100ml of ethanol.
- Cold maceration method was employed for 2 days for proper extraction.
- After 2 days maceration was filtered and carica papaya root extract thus, obtained was dried on water bath.

3.7.3 Hydro-alcoholic extract:

- 10gm of powdered roots of carica papaya was taken into Iodine flask in the ratio of hydro-alcohol.
- Cold maceration method was employed for 2 days for proper extraction.
- After 2 days maceration was filtered and carica papaya root extract thus, obtained was dried on water bath.

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RESULT

4.1 *In-vitro* Anti-urolithiatic analysis by calcium oxalate dissolution method:

4.1.1 For Aqueous extract:

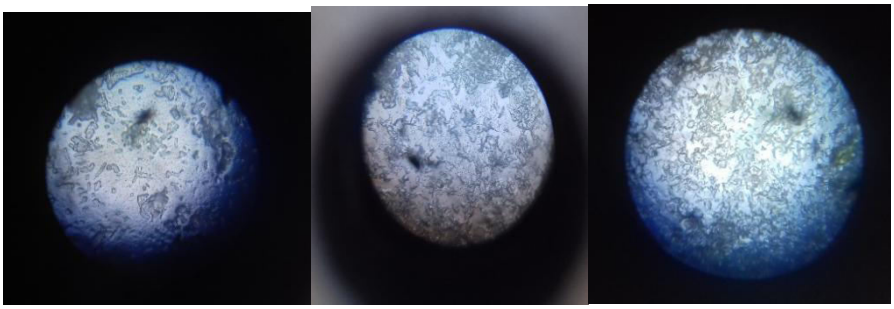

Fig. 4.1 **Fig. 4.2** **Fig. 4.3**

(Microscopic image of dissolved calcium oxalate crystals)

Fig. 4.1 Dilution: For 10mg/ml, Fig. 4.2 Dilution: for 40mg/ml, Fig. 4.3 Dilution: 80mg/ml

4.1.2 For Alcoholic extract:

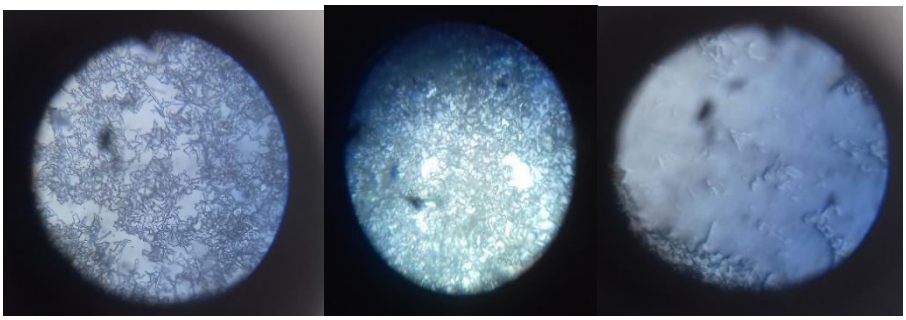

Fig. 4.4 **Fig. 4.5** **Fig. 4.6**

(Most potent)

(Microscopic image of dissolved calcium oxalate crystals)

Fig. 4.4 Dilution: For 10mg/ml, Fig. 4.5Dilution: for 40mg/ml, Fig. 4.6Dilution: 80mg/ml

4.1.3 For Hydro-alcoholic extract:

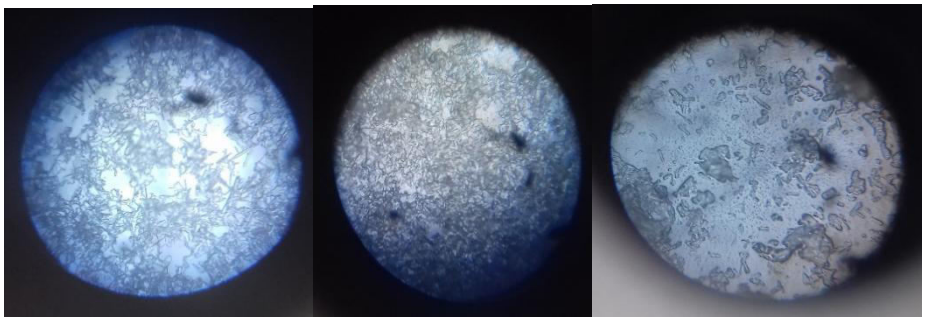

Fig. 4.7 **Fig. 4.8** **Fig. 4.9**

(Microscopic image of dissolved calcium oxalate crystals)

Fig. 4.7Dilution: For 10mg/ml, Fig. 4.8Dilution: for 40mg/ml, Fig. 4.9 Dilution: 80mg/ml

The quantitative analysis by calcium oxalate dissolution method revealed presence of Anti-urolithiatic property in the roots of *carica papaya*. Due to the dissolution of calcium oxalate

crystal occur when the dose of the drug is increased. It means the root extract of carica papaya having a potential to dissolve the crystals of calcium oxalate.The most suitable or significant result comes from the ethanolic extract (Fig. 4.6) of the roots of *carica papaya*. As we increase the dose of crude drug extract the pharmacological action is also increased.

CONCLUSION

The root extract of *Carica papaya* reduces and inhibits the growth of Calcium oxalate stones showing its effect as an antiurolithiatic agent.

It is also seen that the prophylactic effect is more efficient than the curative effect. Therefore, the root extract of *Carica papaya* is useful to prevent the recurrence of urolithiasis as it proved it effect on the early stages of stone development. The mechanism causing this effect is still unspecified, but is possibly related to increased diuresis and lowering of urinary concentrations of stone-forming components.

Supersaturation of urinary salts and crystal retention in urinary tract are the bases for urinary stone formation. Hyperoxaluria and hypercalciuria can be considered as the major risk factors in the pathogenesis of urinary stone formation; evaluation of *carica papaya* is done against calcium oxalate and magnesium ammonium phosphate types of kidney stones, making use of various experimental models of urolithiasis. With regard to reducing oxalate significant levels and have potential antiurolithiatic activity.

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