ISSN:2394-2371
CODEN (USA):IJPTIL

RESEARCH PAPER

# In Vitro Antiurolithic Activity of Ficus Palmata Leaves

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## ABSTRACT

Crude drugs are less efficient with respect to cure of disease but are relatively free from side effects. A number of medicinal plants are evaluated mainly against calcium phosphate types of kidney stones, employing various experimental models of urolithiasis. Most of these studies were preliminary, carried out in animals and are not sufficient for the development of a pharmaceutical product. Still, intensive preclinical and clinical studies are required to evaluate the efficacy and toxicity of these plant products. In the present study the plant *Ficus palmata* was selected for its considerable flavonoidal content and an attempt was made to evaluate anticalcinogenic potential. The aqueous extract of *Ficus palmata* showed dose dependant anticalcinogenic action in demineralization of Calcium Phosphate. Aqueous extract is more potent than methanolic extract at the tested concentrations.

**Keywords:** - Ficus palmate, Urolithiasis

## INTRODUCTION

Urolithiasis is an extremely painful disease that afflicts the human population since ancient times the mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty associated with it.

### Causes

Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease. Oxalate, the major stone-forming constituent, is known to induce lipid peroxidation which causes disruption of the cellular membrane integrity. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. This alters the membrane fluidity, permeability and thereby affects the ion transport across the cellular organelle.

Calcium oxalate is one of the main constituents of deposits in urinary tract. Crystallization of calcium oxalate is of particular interest not only from the theoretical point of view but also because of its biological importance. The exact mechanism of the

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**Article Published: Jan.-March 2019**

initiation of the calcium oxalate stone formation is not completely understood. Factors leading to the nucleation, crystal growth and aggregation of hydrates of calcium oxalate depend not only on the excess of calcium and oxalate concentrations but also on the presence of various foreign substances.

It is the development of stones in the urinary tract, which may lead to pain and bleeding. It is considered the third most common affliction of the urinary tract. In most of the types of stones that are formed, the most frequent are calcium oxalate. As per clinical and epidemiological studies, calcium oxalate followed by calcium phosphate is the most commonly encountered crystalline components found in urolithiasis.

**Treatment [4,7]**

The accepted management of stone disease ranges from observation (watchful waiting) to surgical removal of the stone. Various factors such as size of calculi, severity of symptoms, degree of obstruction, kidney function, location of the stone and the presence or absence of associated infection influence the choice of one type of intervention over the other Stones which are smaller than 5mm have a high probability of spontaneous passage which can take up to 40 days . During this watchful waiting period, patients can be treated with hydration and pain medication However, stones larger than 5mm or stones that fail to pass are treated by interventional procedures Open surgical procedures for the treatment of uretharic stones have gradually disappeared in the last 30 years and have been replaced by minimal invasive techniques such as ESWL or ureteroscopy. ESWL is a noninvasive procedure which uses shock waves to fragment calculi. This technique is the most widely used method for managing renal and urethral stones. However, treatment success rates depend on stone composition, size, properties and location of the stone as well as the instrumentation type and shock frequency .It also needs to be considered that the same forces that are directed at the stones have deleterious effects on surrounding tissues .Damage to almost every abdominal organ system has been reported ,but by far the most common injury is acute renal hemorrhage although its true incidence is unclear and poorly defined Most often renal hemorrhage can be managed conservatively; however, in rare instances the complications are fatal reports of post-ESWL perirenal hemorrhage from less than 1% to greater than 30% .Furthermore, ESWL has been associated with long-term medical effects such as diabetes mellitus and hypertension In addition to ESWL, other procedures such as ureteroscopy (URS) have been developed for removal of urethral stones. The new generations of uteroscopes are flexible, smaller in diameter, stiffer and more durable, and have an improved tip deflection. The major drawback of URS is that it is more invasive than ESWL and the rate of ureteric perforation and structure formation remains around 2 to 4%. In contrast, the major advantage of URS is that it is cheaper and results in higher and faster stone free rates It remains unclear which treatment modality is better than the other and the final decision should be based on the patients preference, on the size and the location of the stone, expertise of the physician and the costs of the procedure. Although there are a few recent reports of beneficial effects of medical treatments in enhancing clearance of stones in the distal ureters, there is still no satisfactory drug to use in clinical therapy, especially for the prevention or the recurrence of stones. In this regard many plants have been traditionally used to treat kidney stones and have been shown to be effective.

Herbal medicines are considered as the basis of therapy throughout the world since the ancient time of and are still widely used. Recognition of their therapeutic uses is still growing, although this varies widely in different countries. Huge segment of inhabitants in developing countries still rely on the conventional practitioners, medicinal plants and herbal medicines for their prime care. During past decades, public curiosity in natural therapies has augmented to a great extent in developed countries, with mounting use of medicinal plants and herbal medicines.

Medicinal plants and derived medicine are widely used in traditional cultures all over the world. Nearly 80% of the world populations depend on herbal and/or alternative medicine for their primary health care.

**Plant profile**

***Ficus palmata*[9]**

**Fig. 1** Leaves of *Ficus palmata*[6]

**Vernacular names**: Wild fig, Punjab fig , Bedu, Timilo,

**Taxonomical classification**

| | |
|---|---|
| **Kingdom** | Plantae |
| **Subkingdom** | Tracheobionta |
| **Super division** | Spermatophyta |
| **Division** | Magnoliophyta |
| **Class** | Magnoliopsida |
| **Subclass** | Hamamelididae |
| **Order** | Urticales |

| | |
|---|---|
| **Family** | Moraceae |
| **Genus** | Ficus |
| **Species** | Palmata |

**Morphology**

Punjab Fig is a deciduous, moderate-sized tree, 6-10 m in height. It is usually seen cultivated as a shrub. Young branches, velvety, often becoming hairless

- **Bark**: smooth, dull, ash gray, can be stripped off with the hand, exposing the white to light-yellow wood underneath; wood, moderately hard.
- **Leaves:** alternate, broad, ovate, membranous, 12.92 cm long, 14.16 cm broad, with a heart-shaped base, and toothed margin (which is rare in figs). Leaves are dark green and rough on the upper surface light green and tomentose on the lower surface.
- **Fruits:** Punjab Fig is one of the tastiest fruits found growing wild in the mid-Himalayan region. It is at par with the cultivated figs in taste and flavour, however, size is rather small in this case. In the hills of India, this fig is eaten largely and is succulent, sweet and pleasant.

**Chemical Constituents [9]**

Ficus species contain flavanoid glycosides, alkaloids, phenolic acids, steroids, saponins, coumarins, tannins, triterpinoids – oleanolic acid, rusolic acid, α- hydroxy ursolic acid, protocatechuic acid, maslinic acid. The nonenzymatic constituents include phenolic compounds, flavanoids, vitamin C. The enzymatic constituents present are ascorbate oxidase, ascorbate peroxidise, catalase, peroxidise. The phenolic compounds present are gallic acid and ellagic acid.

Furanocoumarins that are reported are psoralen, bergapten.[3] β-sitosterol and a new tetracyclic tritepene – glaunol acetate are reported from the leaves, bark and heartwood of F.palmata. Besides ceryl behenate, lupeol, α-amyrin acetate was reported from the stem bark of *F. palmate*.

**Uses [9,10]**

- Edible, Fodder and fuel wood.
- Fruit paste is used in ringworm and skin diseases.
- Ripe fruits are used in dysentery and vomiting.
- Latex is applied to extract spines deeply lodged in the flesh.
- It is used as a part of the diet in the treatment of constipation and diseases of the lungs and urinary bladder.
- The ayurvedic pharmacopoeia recommends the use of bark in lipid disorders and obesity.
- Lignin, the major fibre constituent of the fruit prevented the rise in serum cholesterol levels.

**NEED & PLAN OF THE WORK:**

The kidney stone formation or urolithiasis is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation, and retention within the kidneys. Epidemiological data have shown that calcium oxalate is the predominant mineral in a majority of kidney stones. Among the treatments used are extracorporeal shock wave lithotripsy (ESWL) and drug treatment. Even improved and besides the high cost that imposes, compelling data now suggest that exposure to shock waves in therapeutic doses may cause acute renal injury, decrease in renal function and an increase in stone recurrence. In addition, persistent residual stone fragments and the possibility of infection after ESWL represent a serious problem in the treatment of stones. Furthermore, in spite of substantial progress in the study of the biological and physical manifestations of kidney stones, there is no satisfactory drug to use in clinical therapy. Data from in vitro reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctive therapy in the management of urolithiasis. The present work evaluates the potential usefulness of herbal medicines in the management of urolithiasis.

## METHOD AND RESULTS

### Preparation of extract by cold maceration

**Aqueous extract**

100 gm of the drug was soaked with a little of distilled water in a stainless steel closed container. After an hour 200 ml of water was added to the moistened drug and it was allowed to macerate for 07 days with occasional shaking. After a week liquid was filtered with the help of muslin cloth and the drug material was pressed to liberate more menstrum from the marc. Both the extract was mixed and the liquid was evaporated to get a brown colored aqueous extract. The percentage yield was calculated

**Methanolic extract**

100 gm of the drug was soaked with a little of distilled methanol in a stainless steel closed container. After an hour 200 ml of methanol was added to the moistened drug and it was allowed to macerate for 07 days with occasional shaking. After a week liquid was filtered with the help of muslin cloth and the drug material was pressed to liberate more menstrum from the marc. Both the extract was mixed and the liquid was evaporated to get a green colored methanolic extract. The percentage yield was calculated

Percentage yield of aqueous extract= 09.50%

Percentage yield of methanolic extract=05.40%

**Determination of extractive value [1]**

**Water soluble extractive value**

5gm of dried drug (coarsely powder) was taken and macerated with 100 ml of water closed flask for 24 hrs. Shaking frequently during first 6 hr and allowed to stand for 18 hrs. After 24 hrs the extract was filtered and 30 ml of filtrate was evaporated to dryness in a flat bottom shallow disk. The weight of the residue was noted and the percentage of the water soluble extractive value was calculated with reference to the dried drug used

**Alcohol soluble extractive value**

5gm of dried drug (coarsely powder ) was taken and macerated with 100 ml of absolute alcohol (99.90%) closed flask for 24 hrs. shaking frequently during first 6 hr and allowed to stand for 18 hrs. after 24 hrs the extract was filtered and 30 ml of filtrate was evaporated to dryness in a flat bottom shallow disk. The weight of the residue was noted and the percentage of the alcohol soluble extractive value was calculated with reference to the dried drug used.

**Table No. 4:** Extractive values of *F. palmata*

| S. No. | Type of Extractive value | Extractive value in gram | % Extractive value |
|---|---|---|---|
| 1 | Water soluble | 0.63 | 12.60% |
| 2 | Methanol soluble | 0.21 | 4.01% |
| 3 | Hydroalcoholic | 0.44 | 8.80% |

**Anticalcinogenic activity [5,7]**

Effect of aqueous extract of *F. palmata* leaves was studied *in vitro* homogenous system of initial mineral phase formation for calcium phosphate, its subsequent growth and demineralization.

**For Mineralization**

By employing 5.0 ml system which was prepared by adding 0.5 ml of KH2PO4 (50 mM), 0.5 ml of CaCl2 (50 mM), 2.5 ml of Tris buffer (210 mM NaCl + 0.1 mM tris HCl) and increasing volume of the aqueous extract ranging from 0.2 ml to 0.8 ml by subsequently decreasing the volume of water ranging from 1.3 ml to 0.7 ml. This system was centrifuged at 4500 rpm and precipitates so obtained were dissolved in 5 ml of 0.1 N HCl.

**For Growth**

Firstly 5 ml systems were prepared using above standard protocols then again 5 ml systems were re-grown on the same tubes with the additions of increasing volumes of the extract. Calcium and phosphate were then estimated on the precipitates obtained and dissolved in 0.1 N HCl. In case of control no extract was added.

**For Demineralization**

Again 5 ml system was prepared having no extract added to that and precipitates were obtained. To these precipitates, 2.5 ml of Tris buffer (210 mM NaCl+0.1 mM Tris HCl) and increased 19 volumes of extract ranging from 0.2 ml to 0.8 ml with subsequently reduced volume of water was added and then centrifuged at 4500 rpm for 15 min. Calcium and phosphate were then estimated in supernatant obtained after centrifugation.

**The $HPO_4^{-2}$ ions were estimated by the following method [8]**

10 % ascorbic acid solution was prepared by mixing 10gm ascorbic acid in 100 ml water. 2.5% ammonium molybdate solution was prepared by mixing 2.5 gm in 100 ml water. 6 N Sulphuric acid solutions were prepared by mixing 18 ml conc. Sulphuric acid and water up to 108 ml. Two volume of water and one volume of each of above sample was mixed, this is known as Reagent C. 1ml of test sample and 1ml of Reagent C was mixed .This mixture was incubated for 2hours at 37 $^0$C. Absorbance was taken at 820 nm in U.V. spectrophotometer. Absorbance 0.240 = 0.01 m. mol phosphate ion conc.

**Percent inhibition of mineral phase in the presence of test plant aqueous extract was calculated as:**

% inhibition=(C-T /C)*100, where T is the concentration of HPO42- ion of the precipitate formed in test having plant aqueous extract ranging from 0.2 ml to 0.8ml in the assay system and C is the concentration of HPO42- ion of the precipitate formed in control system which had distilled water and no extract.

**For Mineralization**

**Table No.6:** Effect of *Ficus palmata* on mineralization of Calcium Phosphate Crystals

| S. No. | Conc. of test plant extract | Absorbance | Concentration of phosphate ion (m.mol/ml) | % Inhibition |
|---|---|---|---|---|
| **1** | 0 | 0.101 | 0.210 | 0 |
| **2** | 0.2 | 0.109 | 0.226 | -7.61 |
| **3** | 0.4 | 0.215 | 0.447 | -112.85 |
| **4** | 0.6 | 0.186 | 0.386 | -83.80 |
| **5** | 0.8 | 0.425 | 0.884 | -320.95 |

**For Growth Phase**

**Table No. 7:** Effect of *Ficus palmata* on growth of Calcium Phosphate Crystals

| S. No. | Conc. of test plant extract(ml/5ml) | Absorbance | Concentration of phosphate ion ( m mol/ml) | % Inhibition |
|---|---|---|---|---|
| **1** | 0 | 0.125 | 0.288 | 0 |
| **2** | 0.2 | 0.210 | 0.436 | -51.38 |
| **3** | 0.4 | 0.351 | 0.730 | -153.47 |
| **4** | 0.6 | 0.389 | 0.809 | -180.90 |
| **5** | 0.8 | 0.401 | 0.834 | -189.58 |

**For Demineralization Phase**

**Table No. 8:** Effect of *Ficus palmata* on demineralization of Calcium Phosphate crystals

| S. No | Conc. of test plant extract (ml/5ml) | Absorbance | Conc. of phosphate ion(mmol/ml) | % Inhibition |
|---|---|---|---|---|
| 1 | 0 | 0.241 | 0.555 | 0 |
| 2 | 0.2 | 0.282 | 0.586 | 5.31 |
| 3 | 0.4 | 0.460 | 0.956 | 41.95 |
| 4 | 0.6 | 0.670 | 1.393 | 60.15 |
| 5 | 0.8 | 1.201 | 2.501 | 77.80 |

Determination of extractive values is done for that material for which no chemical or biological assay method exist. According to Indian Pharmacopoeia 1996 and British Pharmacopoeia 1980, the determination of water soluble and alcohol soluble extractives is used as a means of evaluating crude drugs which are not readily estimated by other means. The water, alcohol and ether soluble extractive values for dried whole plant were found to be 10%, 2.0 % and 0.12% respectively. These values indicated the presence of polar compounds in the drug which exhibited the anticalcinogenic activity. Ash value indicates contamination, substitution, adulteration, or carelessness in preparing the drug or drug combinations for marketing. These values were found to be reasonably low for the sample of drug used. Total ash value of plant material gives an idea about the amount of minerals and earthy materials present in the plant material. Total ash for the drug sample was found 0.57 % were as the acid insoluble and water soluble ash were found to be 11.60% and 11.40 % respectively

**Anticalcinogenic activity**

As evident from above tables the aqueous extract of *ficus palmata* showed dose dependant anticalcinogenic action in demineralization of Calcium Phosphate.

Summary & Conclusion

. In the present study the plant *Ficus palmata* was selected for its considerable flavonoidal content and an attempt was made to evaluate anticalcinogenic potential. The aqueous extract of *Ficus palmata* showed dose dependant anticalcinogenic action in demineralization of Calcium Phosphate. Aqueous extract is more potent than methanolic extract at the tested concentrations. However, to establish *Ficus palmata* as an anticalcinogenic drug studies with other methods of evaluation of Urolithiatic action needs to be undertaken.

Further, bioactivity guided isolation of the active compound from the aqueous extract can also help in standardization of its anticalcinogenic activity.

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**Cite this article as:**

Kumar B., Tiwari Y., Singh A., Kumar V. In Vitro Antiurolithic Activity of Ficus Palmata Leaves. Int. J. Pharm. Technol. Biotechnol. 2019; 6(1): 01-09.