Original Research Article

EVALUATION OF ANTI-UROLITHIASIS ACTIVITY BY NUCLEATION ASSAY

ABSTRACT

Many causes, including the efficiency of plant medicines and their lower side effects when compared to contemporary medications, have contributed to the current rebirth of plant therapies. Given the current situation, basic scientific research on the therapeutic herbs utilized in indigenous systems is urgently needed. The purpose of this review is to provide information on current developments in the study of medicinal plants recognized for their antiurolithiatic properties. This could assist researchers in determining and creating suitable lead compounds or plant products that are advantageous in the treatment of urolithiasis.

Keywords: Kidney stones, Calcium oxalate crystals, Nucleation, Cystone.

INTRODUCTION

The disorder known as urolithiasis is described as happening when these stones leave the renal pelvis and travel through the bladder, urethra, and ureters to the rest of the urine collecting system.

Kidney stones are the result of a number of physicochemical processes that include supersaturation, nucleation, development, aggregation, and retention inside the kidneys. In developed nations, 10–12% of people suffer with urinary stones.[1] One of the most common and ancient illnesses in human history is kidney stone production. Urinary calculi have been found in Egyptian mummies dating back to 4000 BC and in the tombs of North American Indians dating from 1500–1000 BC. In India, between 3000 and 2000 BC, early Sanskrit texts mention the production of stones. Current treatments include extracorporeal shock wave lithotripsy (ESWL), percutaneous techniques, and surgical excision are too costly for the typical individual to afford. These treatments frequently have recurrence as a side effect, thus patients need to have close follow-up for a number of years. The recurrence rate may be decreased by pharmacotherapy. It is important to employ plant products that traditional medical systems claim to have therapeutic benefits.[2]

Seventy-five to ninety percent of cases of stones are calcium-containing, namely calcium oxalate monohydrate, calcium oxalate dihydrate, and basic calcium phosphate. 10%–15% is made up of magnesium ammonium phosphate (Struvite), 3%–10% is uric acid, and 0.5–1% is cystine (4-6). The most common types of stones are usually either calcium oxalate or magnesium ammonium phosphate. Urinary stones are said to benefit from the use of plants of the "Pashanabheda" category in India's Ayurvedic medical system. A class of plants having diuretic and antiurolithiatic properties is known by the Sanskrit title "Pashanabheda" (Pashana = stone, Bheda = break).[1]

Urinary tract infections, diets heavy in oxalates and calcium, vitamin abnormalities like excess D or vitamin A deficiency, and metabolic illnesses including hyperthyroidism, cystinuria, gout, intestinal dysfunction, etc. are some of the causes of insufficient urine flow.are the main causes of stone formation. Urolithiasis, or the production of kidney stones, is a complicated process brought on by an imbalance between the kidneys' promoters and inhibitors. Urine output is what influences the creation of stones, which explains the concentration.urine pH, the concentration of a certain component, and urinary tract infections or damage.[3]

classification of urolithiasis

Urolithiasis can be classified as:

1. Calcium Oxalate
2. Uric Acid
3. Struvite
4. Cystine

1) CALCIUM OXALATE
   - Combining oxalate and calcium in urine results in the most prevalent kind of kidney stone formation.
   - A lack of calcium and fluids, along with other factors, may aid in their development.

2) URIC ACID
   - This kind of kidney stone is also frequently occurring.
   - Foods high in purines, a naturally occurring chemical component, include shellfish and organ meats.
   - Consuming a lot of purines increases the body's production of monosodium urate, which might cause kidney stones in some situations.
   - Usually, these kinds of stones originate within families.

3) STRUVITE
   - These stones are less prevalent and are brought on by upper urinary tract infections.
   - Women are more likely to get struvite stones, which are brought on by particular types of UTIs.
   - These stones frequently enlarge and expand quickly, sometimes taking up the entire kidney.
   - Oftentimes, these stones grow rapidly, often filling the entire kidney.

signs and symptoms of urolithiasis

Kidney stones, which are solid masses composed of microscopic crystals, are formed as a result of urolithiasis. It is possible for the kidney or ureter to contain one or more stones concurrently. You might be passing a kidney stone if you've ever had sudden, intense pain that comes and goes in your abdomen or one side of your back.

- Experiencing pain in your side or lower back.
- Experiencing nausea in addition to vomiting.
- Detectable blood in your pee.
- Having discomfort when urinating.
- Having trouble urinating.
- Experiencing increased frequency of urination.
- Fever or chills.
- Having hazy or unpleasant-smelling urine.

pathogenesis of urolithiasis

The development of urinary stones is caused by many processes. Bacterial metabolism causes infection stones, on the other hand, exceeding supersaturation is the source of cystine calculi or uric acid calculi (i.e., free stone production).

Surprisingly, the production of the most prevalent component, the calculi containing calcium, is more intricate and yet little understood.

It appears from recent data that stone can form both freely and fixedly.

- Inhibitors of stone formation:

  When there is a lack of certain minerals, such as citrate, magnesium, ureapontin, and nephrocalcin, which ordinarily inhibit crystallisation in the urine, stones might form.(that prevent calcium-containing crystals from growing, aggregating, or nucleating)
- Supersaturation of urine:

When the urine gets supersaturated with one or more calculogenic (crystal-forming) substances, a seed crystal may form by nucleation.

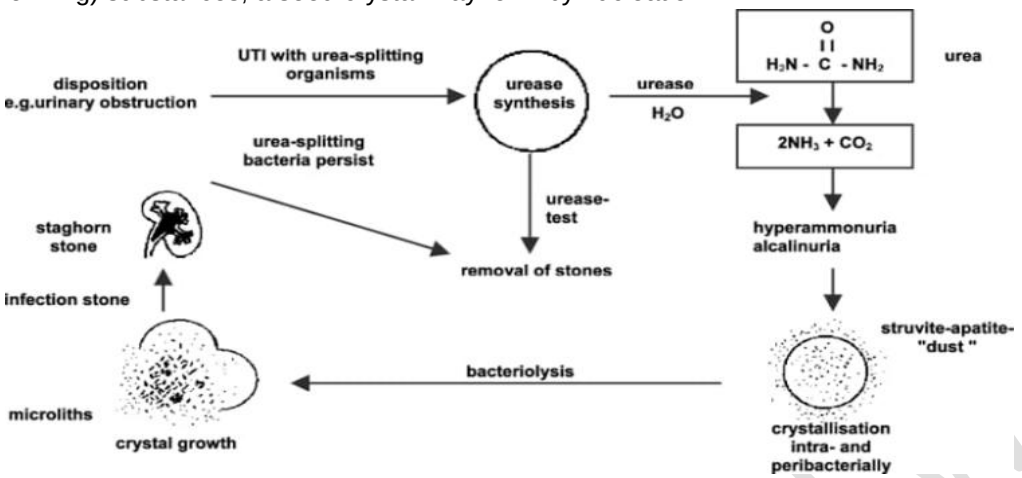

Fig . 1Pathogenesis of urolithiasis

PLANT PROFILE

PHYLLANTHUS ACIDUS

PLANT INTRODUCTION:

Botanical name :*Phyllanthus acidus*
Family :Phyllanthaceae.
Indian Name :Usiri, Holphali, Leyoir.
Habitat : It is small deciduous tree found in moist tropical and subtropical coastal woodlands and disturbed sites.
Parts Used : Fruit.
Phytochemical constituents : Alkaloids, flavonoids, tannins, glycosides, lignin, terpenes, sterols.

BOTANICAL CLASSIFICATION:

Phyllanthus acidus belongs to the family of “Phyllanthaceae.”

CLASSIFICATION:

Kingdom : Plantae
Division :Tracheophyta
Class : Magnoliopsida
Order :Malpighiales
Family :Phyllanthaceae
Genus : Phyllanthus
Species : P. acidus

TINOSPORA CORDIFOLIA

PLANT INTRODUCTION:

Botanical name :*Tinospora cordifolia*
Family : Menispermaceae.
Indian Name :Guduchi, Giloy.
Habitat :Dispersed throughout India's tropical regions, which are situated 1200 metres above sea level from Kumaon to Assam. originally from India, Myanmar, and SriLanka.
Parts Used : Leaves.
Phytochemical constituents : Alkaloids, steroids, glycosides, tannins, flavonoids

BOTANICAL CLASSIFICATION:

Tinospora cordifolia belongs to the family of “Menispermaceae.”

CLASSIFICATION:

Kingdom : Plantae
Division :Tracheophyta
Class : Magnoliopsida
Order :Ranunculales
Family :Menispermaceae
Genus :Tinospora
Species : T. cordifolia

PSIDIUM GUAJAVA

PLANT INTRODUCTION:

Botanical name	:*Psidium guajava*
Family	: Myrtaceae.
Indian Name	: Amrudh, Amarood, Peyara.
Habitat	: Guava is successfully grown under tropical and subtropical climate. The quality of fruit is better in ares having distinct winters. Guava tolerates drought, protective irrigation facilities are required.
Parts Used	: Fruits and Leaves.
Phytochemical constituents	: The fruit contains Saponins, Oleanolic acid, Lyxopyranoside, Araboyranoside, and Flavonoids.

BOTANICAL CLASSIFICATION:

Psidium guajava belongs to the family of “Myrtaceae.”

CLASSIFICATION:

Kingdom	: Plantae
Division	:Mangnoliophyta
Class	: Magnoliopsida
Order	: Myrtales
Family	:Myrtaceae
Genus	: Psidium L
Species	:P.guajava

Plants And Plant Products WithAntiurolithiatic Activity

Urinary calculi in the kidney and bladder have been effectively dissolved in clinical settings by the commercialised composite herbal formulations Cystone (Himalaya Drug Company, India), Calcuri (Charak Pharmaceuticals, Bombay, India), and Chandraprabha bati (Baidyanath, India).Cyperusrotundus, Tinospora cordifolia, Psidium gujava, and Phyllanthus acidus are a few herbal plants that have urolithiatic activity.

PHYTOCHEMICAL TESTS

FLAVONOIDS

Shinoda test: A piece of metallic magnesium was combined with 1 millilitre of extract and warm the test tube in a water bath while adding two drops of HCl. The occurance of orange, red /violet precipitate indicates presence of flavonoids.To 1ml aqueous extract add 1ml 10% lead acetate results in yellow precipitate indicates presence of flavonoids.

SAPONINS

Foam test: Take 3ml of each extract and add 2ml of distilled water in test tube to dilute it. Now shake the mixture vigorously. The formation (or) occurance of foam results test /The formation of foam indicates saponins.

TANNINS

Take 2ml of each extract in each test tubes and boil them for 2mins and allow the test tubes to cool. Add three drops of ferric chloride solution to each extract after it has cooled. The colour changes to dark blue results presence of tannins.

METHODS

PREPARATION OF EXTRACTS

The selected plant materials were separately extracted successively with selected solvent with increase order of polarity using suitable extraction process and preliminary phytochemical study was performed on various liquid extracts.

Fresh plant material was taken from young, mature plants of Tinospora cordifolia, Phyllanthus acidus, and Psidium gujava, and it was verified. Following verification, the plant components were gathered in bulk, rinsed with distilled water, and then cleaned under running tap water to get rid of any remaining dirt. After being shade dried, the plant components were separately ground into a coarse powder using a mechanical grinder.

- **Preparation of Extract**

Using a soxhlet extractor, the dried powdered plant components (500 g each) were extracted individually and progressively with methanol and water. At every stage of the extraction procedure, the extraction period was set at five hours for each solvent. Following the extraction process, the dried plant material's extractive value was ascertained. Following the extraction of the filtrate, it was put into petri plates that had been weighed. By allowing the filtrate to fully evaporate the solvent, the resulting extracts were concentrated until they were completely dry. Using the following formula, the extractive value in percentage was computed and recorded.

Extractive value (%) = Weight of dried extract/Weight of plant material X 100

NUCLEATION ASSAY

The inhibitory activity of the extracts on the nucleation of CaOx crystals was determined by a spectrophotometric assay. Crystallisation was initiated by adding calcium chloride (4 mmol/L) and

sodium oxalate (50 mmol/L) solutions to artificial urine, both prepared in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 and 37 °C. The rate of nucleation was determined by comparing the induction time of crystals (time of appearance of crystals that reached a critical size and thus became optically detectable) in the presence of the extract and that of the control with no extract. The absorbance (optical density, OD)was recorded at 620 nm, and the percentage inhibition calculated as (OD (experimental)/OD (control))/100.

Formula for the calculation of Nucleation assay:

$$I\% = (AC - AS)/AC \times 100$$

RESULT AND DISCUSSION

The antiultrathiatic qualities of many herbal folk remedies make them indispensable for preventing disease. This article aims to highlight a potent native herb that is used to cure kidney stones.Additionally, herbs enhance renal function and control oxalate metabolism, both of which lower the risk of renal calculi recurring.[3] Numerous herbs themselves have anti-crystallization properties. Additionally, the antioxidant properties of the herbs aid in avoiding renal cell damage from urolithiatic disease. Even though the use of herbal medicine is growing and is quite common, further research is needed to fully understand the pathophysiology of disease and the mechanism of action of herbal medicines in order to develop a litholytic agent that is both safe and effective. [4]

Table: 1 Phytochemical tests of herbal plants

Test	Flavonoids	Alkaloids	Glycosides	Tannins	Saponins	Phenolics
Phyllanthus acidus(Fruit)	+	-	-	+	+	-
Tinosporacordifolia(Leaf)	+	-	-	+	+	-
Psidium gujava(Fruit)	+	-	-	+	+	-
Psidium gujava(Leaf)	+	-	-	+	+	-

Table: 2 The comparative study of various herbal plants with standard drug cystone

Sample	Concentration(ug)	Absorbance@620nm	Percentage Inhibition
Methanolic fruit extract of Phyllanthus acidus	100ug	1.17	40%
Methanolic leaf extract of Tinospora cordifolia	100ug	1.14	42%
Aqueous leaf extract of Tinospora cordifolia	100ug	1.12	43%
Methanolic leaf extract of Psidium gujava	100ug	1.11	43%
Aqueous leaf extract of Psidium gujava	100ug	1.09	44%
Methanolic fruit extract of Psidium gujava	100ug	1.15	41%
Aqueous fruit extract of Psidium gujava	100ug	1.05	46%
Cystone (Standard drug)	100ug	1.7	62%

CONCLUSION

Numerous plants are said to be helpful in treating urinary stones in the extensive Ayurvedic literature; nevertheless, many more plants still need to be used for their pharmacological effects. Nothing traditional is available for the treatment of urinary stones, despite a great deal of research into the mechanisms underlying stone formation, nutritional management, evaluation of medicinal herbs, and other agents.As this review shows, many therapeutic herbs are tested primarily using different experimental models of urolithiasis against kidney stone types caused by calcium oxalate and magnesium ammonium phosphate. Most of these studies were exploratory, animal-based, and not sufficiently conclusive to justify the development of a pharmacological product. While they might not completely replace these methods, plant materials and their lead component derivatives might undoubtedly aid in lowering the rate at which renal calculi reoccur.[2] There is growing interest in the use of herbal remedies for illness prevention and treatment due to the drawbacks of modern medicine paired with the superiority and efficacy of activity supplied by natural elements in herbs.[3]

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