JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

TO STUDY IN-VITRO UROLIATHIASIS ACTIVITY OF *DENDROPHTHOE FALCATA.*

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ABSTRACT

The powder of plant *Dendrophthoe falcata* was selected for present study, powder of whole plant extracted with Alcohol, Pet ether, Water for 24hrs by using Soxhlet apparatus successively with various solvent are removed under reduced pressure. Extract is concentrated to dryness at controlled temp. Dried powder drug are evaluated for amount of drug extracted during process of extraction and % of extraction of drug in various solvent are also calculated. Then preliminary phytochemical screening was performed and microscopic and characters were studied compound, microscopic characters also studied. Dried powdered drug contains only Flavonoid Gallic acid, Pentacyclic triterpenoid, saponin glycoside.

Microscopic study also performed for fresh leaves using Sudan Red and Phloroglucinol: HCl. Natural products from medicinal plants, either as pure compound or as extracted out, provide opportunities for new herbal drugs because of unmatched availability of chemical diversity. Herbal preparation contain various bioactive compounds Research have revealed many chemical constituents isolated from this plant to exhibit several biological activities present study aims at studying the antiurolithiatic activity from the ethanolic extract obtained from the powder drug of Dendrophthoe Falcata using in-vitro model for study. From the above study it was found that it shows the urolithiasis activity.

Keywords: - calcium oxalate, crystallisation, Dendrophthoe falcate, urolithiasis.

1.INTRODUCTION

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

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. Crystallisation 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 initiation of the calcium oxalate stone formation is not completely understood. Factors leading to the nucleation, crystal growth and aggregation of various hydrates of calcium oxalate depend not only on the excess of calcium and oxalate concentrations but also on the presence of various foreign substances.[1,6,7].

1.1 Urolithiasis:[12,16]

The formation of stone in the urinary system, i.e., in the kidney, ureter, and urinary bladder or in the urethra is called urolithiasis. 'Urolithiasis' = ouron (urine) and lithos (stone).

1.1.1 Types of Urolithiasis:

The stone type is named after its mineral composition. The most common stones are struvite (magnesium ammonium phosphate), calcium oxalate, urate, cystine and silica.

a) Calcium oxalate stones:

The most common type of kidney stones worldwide contains calcium. For example, calcium-containing stones represent about 80% of all cases in the United States; these typically contain calcium oxalate either alone or in combination with calcium phosphate in the form of apatite or brushite. Factors that promote the precipitation of oxalate crystals in the urine, such as primary hyperoxaluria, are associated with the development of calcium oxalate stones. The formation of calcium phosphate stones is associated with conditions such as hyperparathyroidism and renal tubular acidosis. Calcium oxalate stones appear as 'envelopes' microscopically. They may also form 'dumbbells'. Calcium oxalate crystals in the urine are the most common constituent of human kidney stones, and calcium oxalate crystal formation is also one of the toxic effects of ethylene glycol poisoning. Hydrated forms of the compound occur naturally as three mineral species: whewellite (monohydrate, known from some coal beds), weddellite (dihydrate) and a very rare trihydrate called ca oxalates. Most crystals look like a sided prism and often look like a pointed picket from a wooden fence. More than 90% of the crystals in urine sediment will have this type of morphology. These other shapes are less common than the sided prisms, however it is important to be able to quickly identify them in case of emergency.

b) Struvite stones:

About 10–15% of urinary calculi are composed of struvite (ammonium magnesium phosphate). Struvite stones (also known as "infection stones", urease or triple-phosphate stones), form most often in the presence of infection by urea-splitting bacteria. Using the enzyme urease, these organisms metabolize urea into ammonia and carbon dioxide. This alkalinizes the urine, resulting in favourable conditions for the formation of struvite stones. Proteus mirabilis, Proteus vulgaris, and Morganella morganii are the most common organisms isolated; fewer common organisms include Ureaplasma urealyticum, and some species of Providencia, Klebsiella, Serratia, and Enterobacter. These infection stones are commonly observed in people who have factors that predispose them to urinary tract infections, such as those with spinal cord injury and other forms of neurogenic bladder, ileal conduit urinary diversion, vesicoureteral reflux, and obstructive uropathies. They are also commonly seen in people with underlying metabolic disorders, such as idiopathic hypercalciuria, hyperparathyroidism, and gout. Infection stones can grow rapidly, forming large calyceal staghorn (antler-shaped) calculi requiring invasive surgery such as percutaneous nephrolithotomy for definitive treatment. Struvite stones (triple phosphate/magnesium ammonium phosphate) have a ‘coffin lid' morphology by microscopy.

c) Uric acid stones:

About 5–10% of all stones are formed from uric acid. People with certain metabolic abnormalities; including obesity may produce uric acid stones. They also may form in association with conditions that cause hyperuricosuria (an excessive amount of uric acid in the urine) with or without hyperuricemia (an excessive amount of uric acid in the serum). They may also form in association with disorders of acid/base metabolism where the urine is excessively acidic (low pH), resulting in precipitation of uric acid crystals. A diagnosis of uric acid urolithiasis is supported by the presence of a radiolucent stone in the face of persistent urine acidity, in conjunction with the finding of uric acid crystals in fresh urine samples. These patients also have a tendency to form urate stones. Urate stones are especially common after colon resection. Uric acid stones appear as pleomorphic crystals, usually diamond-shaped. They may also look like squares or rods which are polarizable. Patients with hyperuricosuria can be treated with allopurinol which will reduce urate formation. Urine alkalinization may also be helpful in this setting. Patients with hyperuricosuria can be treated with allopurinol which will reduce urate formation. Urine alkalinization may also be helpful in this setting.

d) Cystine stones:

Cystine kidney stones are due to cystinuria, an inherited (genetic) disorder of the transport of an amino acid (a building block of protein) called cystine that results in an excess of cystine in the urine (cystinuria) and the formation of cystine stones. Cystinuria is the most common defect in the transport of an amino acid. Although cysteine is not the only overly excreted amino acid in cystinuria, it is the least soluble of all naturally occurring amino acids.

Cystine tends to precipitate out of urine and form stones (calculi) in the urinary tract. Small stones are passed in the urine. However, big stones remain in the kidney (nephrolithiasis) impairing the outflow of urine while medium-size stones make their way from the kidney into the ureter and lodge there further blocking the flow of urine (urinary obstruction). Obstruction of the urinary tract puts pressure back up on the ureter and kidney. Causing the ureter to widen (dilate) and the kidney to be compressed. Obstruction also causes the urine to be stagnant (not moving), an open invitation to repeated urinary tract infection. The pressure on the kidneys and the urinary infections result in damage to the kidneys. The damage can progress to renal insufficiency and end-stage kidney disease, requiring renal dialysis or a transplant.

The stone are responsible for all the signs and symptoms

of cystinuria, including:

• Haematuria -- blood in the urine

• Flank pain -- pain in the side, due to kidney pain

• Renal colic - intense, cramping pain due to stones in the

urinary tract

• Obstructive uropathy -- urinary tract disease due to

obstruction

• Urinary tract infections

Silicate stones or drug induced stones

Very rarely, stones can form as a result of taking certain medications or herbal products and the subsequent build-up of chemicals from those products in the urine. Some of these are Loop diuretics, Acetazolamide, Topiramate, Zonisamide, Laxatives (when abused), Ciprofloxacin, Sulpha medications, Triamterene, Indinavir, Ephedrine, Guaifenesin, and products containing silica.

1.1.2 Causes of urolithiasis

Dietary factors that increase the risk of stone formation include low fluid intake and high dietary intake of animal protein, sodium, refined sugars, fructose and high fructose corn syrup, oxalate, grapefruit juice, apple juice, and cola drinks. Stone formation commonly occur due to inadequate urinary drainage, foreign bodies in urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities like vitamin A deficiencies, excess vitamin D, and metabolic diseases like hyperthyroidism, cystinuria, gout, intestinal dysfunction etc. Calcium oxalate is considered as main constituent in the renal calculi.

a) Calcium

Calcium is one component of the most common type of human kidney stones, calcium oxalate. Unlike supplemental calcium, high intakes of dietary calcium do not appear to cause kidney stones and may actually protect against their development. This is perhaps related to the role of calcium in binding ingested oxalate in the gastrointestinal tract. As the amount of calcium intake decreases, the amount of oxalate available for absorption into the bloodstream increases; this oxalate is then excreted in greater amounts into the urine by the kidneys.

In the urine, oxalate is a very strong promoter of calcium oxalate precipitation, about times stronger than calcium.

Another electrolyte

b) Vitamins

Despite a widely held belief in the medical community that ingestion of vitamin C supplements is associated with an increased incidence of kidney stones; the evidence for a causal relationship between vitamin C supplements and kidney stones are inconclusive. While excess dietary intake of vitamin C might increase the risk of calcium oxalate stone formation, in practice this is rarely encountered. The link between vitamin D intake and kidney stones is also tenuous.

c) Other

There are no conclusive data demonstrating a cause-and effect relationship between alcohol consumption and kidney stones. However, some have theorized that certain behaviours associated with frequent and binge drinking can lead to systemic dehydration, which can in turn lead to the development of kidney stones.

- **Supersaturation of urine**

When the urine becomes supersaturated (when the urine solvent contains more solutes than it can hold in solution) with one or more calculogenic (crystal-forming) substances, a seed crystal may form through the process of nucleation. Heterogeneous nucleation (where there is a solid surface present on which a crystal can grow) proceeds more rapidly than homogeneous nucleation (where a crystal must grow in liquid medium with no such surface), because it requires less energy. Adhering to cells on the surface of a renal papilla, a seed crystal can grow and aggregate into an organized mass. Depending on the chemical composition of the crystal, the stone-forming process may precede more rapidly when the urine pH is unusually high or low. Supersaturation of the urine with respect to a vasculogenic compound is pH-dependent. For example, at a pH of 7.0, the solubility of uric acid in urine is 158 mg/100 ml. reducing the pH to 5.0 decreases the solubility of uric acid to less than 8 mg/100 ml. The formation of uric

acid stones requires a combination of hyperuricosuria (high urine uric acid levels) and low urine pH; hyperuricosuria alone is not associated with uric acid stone formation if the urine pH is alkaline. Supersaturation of the urine is a necessary, but not a sufficient, condition for the development of any urinary calculus. Supersaturation is likely the underlying cause of uric acid and cystine stones, but calcium-based stones (especially calcium oxalate stones) may have a more complex etiology.

1.2 Plant profile:

Dendrophthoe falcata is also known as "vanda" in the Ayurveda it is known as perennial climbing woody parasitic plant. It is hemiparasitic whose whole plant is used in indigenous system of medicinal agent like cooling, bitter, astringent, aphrodisiac, narcotic, and diuretic, useful in pulmonary tuberculosis, asthma, menstrual disorder, swelling wound and ulcer. Leaf paste is used in skin disease. Leaf paste is applied on boils, setting dislocated bones and extracting pus. The plant has been scientifically proved to have antilithiatic, diuretic, cytotoxic and immunomodulatory activities. A large, bushy evergreen, parasitic plant with grey bark.

Fig1: Dendrophthoe Falcata (Loranthaceae)

1.2.1 Taxonomical classification:[13,14]

- ***kingdom:*** *Plantae*
- ***Order:*** *Santalales*
- ***Family:*** *Loranthaceae*
- ***Genus:*** *Dendrophthoe*
- *Species: D. falcata*

1.2.2 **Synonyms**: *Loranthus amplexifolius Desr., Loranthus bicolour Roxb, Loranthus falcatus L.F., Loranthus longiflorus Desr*

- *Sanskrit: Vrksadani, Bandaka, Vrksaruha, Samharsa.*
- *English: Mistletoe*

- *Gujrathi: Baando*
- *Hindi: bandaa*
- *Kannada: Bandhulu, Bandanike*
- *Malayalam: Itil, Ittikkanni*
- *Marathi: Baandagul*
- *Oriya: Vrudhongo*
- *Punjabi: Pulluri*
- *Tamil: Jiddu, Baadanikaa*
- *Telugu: Jeevakumu*

1.2.3 Growth and distribution:[11,13,14,17]

Dendrophthoe falcata (L.F.) Ettingsh is a perennial climbing woody parasitic plant. It is indigenous to tropical region especially in India, Srilanka, Thailand, China, Australia, Bangladesh, Malaysia and Myanmar. In India it is widely distributed throughout upto 900m.

Fig2: Dendrophthoe falcata growing on host plant.

1.2.4 Morphology:[11,13]

It is evergreen shrub, usually aerial hemiparasitic or other seed plants; stems much branched, often jointed. Leaves 7.6-25.4 cm long and 1.3-12.7cm wide, alternate or sub opposite, petioles flattened above and rounded beneath, inflorescence racemose and spicate, subumbellate, sometimes pubescent. Flowers 2.5-10.2cm long, brightly coloured (red-orange), regular and bisexual, bracteates often 2 or more bracteoles, stamens are many, anthers basified or dorsifixed, ovary inferior and single celled usually without distinct planate, style short or long,

stigma simple. Fruit a 1-seeded berry or drupe with fleshy pericarp and often viscid mesocarp. Seed solitary, without distinct testa, albumen fleshy, and about 1.3cm in diameter.

1.2.5 Chemical constituent:[11,13,14,15]

Leaves: leaves contain flavonoids such as ***quercetin, quercitrin; Tannins*** comprising of ***gallic*** and ***chebulinic acid.***

Stem: Young shoots contain nearly 10 percent ***tannins*** and the stem contain ***Beta-amyrin-o-acetate, Oleanolic acid*** its ***methyl ester acetate, beta-sitosterol and stigmasterol.***

Root: Catechin and ***leucocyanidin*** in the bark

- ***Flavonoids from dendrophthoe falcata parasitic on different hosts***

1.***Murraya koeinnii (Rutaceous):*** Quercetin, Kaempferol, Queretagetin, Quercitrin, hyperoside.

2.Nerium indicum (Apocyanaceae): Quercetin, Quercitrin, myricetin, myricitrin, meratin.

3.Punica granatum (punicaceae): Quercetin, Quercitrin, myricetin, hyperoside and acyl xyloside of quercetin

4.Mangifera indica (Anacardiaceous): Quercetin, Kaempferol, quercitrin, hyperoside and rutin.

5.Scolopia cremate(bixaceae): Quercetin, hyperoside and acyl xyloside of quercetin

6.Albizzia Iebbeck (Fabaceae): Quercetin, Kaempferol, quercitrin, hyperoside and acyl xyloside of quercetin

1.3 Therapeutic uses:

1.Diuretic activity.

2.Wound healing.

3.Antimicrobial.

4.Antioxidant activity.

5.Cytotoxic and immunomodulatory activity.

6.Antitumor.

7.Male contraceptive activity.

8.Antinociceptive activity.

9.Hepatoprotective activity.

10.Antihyperlipidemic activity.

11.Antidiuretc activity.

1.4 Formulation studies:[13]

As tablet binder: Tablets were prepared with D. falcata mucilage and evaluated for tablet characteristic. Wet granulation technique was used for the preparation paracetamol granules. The tablets binder concentration used in formulation were 2, 4, 6 and 8% w/w tablet were compressed to hardness at about 6.6 to 6.9 kg/cm^2.The evaluation of tablet showed 0.98 to 0.53% friability, 10 to 17% min disintegration time and more optimum results as tablet binder. The dendropthoe falcata mucilage was found to be useful for preparation of uncoated tablet dosage form.

Material and Methods

Equipment required:

Soxhlet extractor, glassware

chemicals required:70% ethanol, Dragendroff's reagent, Mayer's reagent, Sulphuric acid, Lead acetate solution, Sodium hydroxide, Bromine water.

Cystone (standard drug)*:* *Himalaya Cystone* (tablets 60) was obtained from a local pharmacy 10g of peeled and crushed tablet in 100ml of filtered Millipore water was kept on magnetic stirrer for 40 min and used fresh with required dilution.

Collection of plant:

The dendrophthoe falcata Linn. plant powder was ordered online from an ayurvedic store the powder was stored in dry state and then was further used for extraction.

Preparation of plant extract: The powder was packed in the pockets made with the filter paper and placed in the Soxhlet apparatus and extracted with ethanol for 8 hours and temperature was maintained at 60 degree C Throughout extraction process. The extract was then collected and solvent was evaporated under vaccum. The resulting ethanolic extract was stored and subjected to further phytochemical study.

Fig 3: Soxhlet extraction

The powder plant Dendrophthoe falcata were selected for present study; the plant powder was obtained from online herbal cart. Then powdered then extracted with Alcohol, Pet ether, Water for 24hrs by using Soxhlet apparatus successively with various solvent are removed under reduced pressure. Extract is concentrated to dryness at controlled temp.

Phytochemical analysis:

The ethanolic extract of Dendrophthoe falcata were subjected to qualitative phytochemical tests for different constituents such as Flavonoids, tannis, saponins and alkaloids.

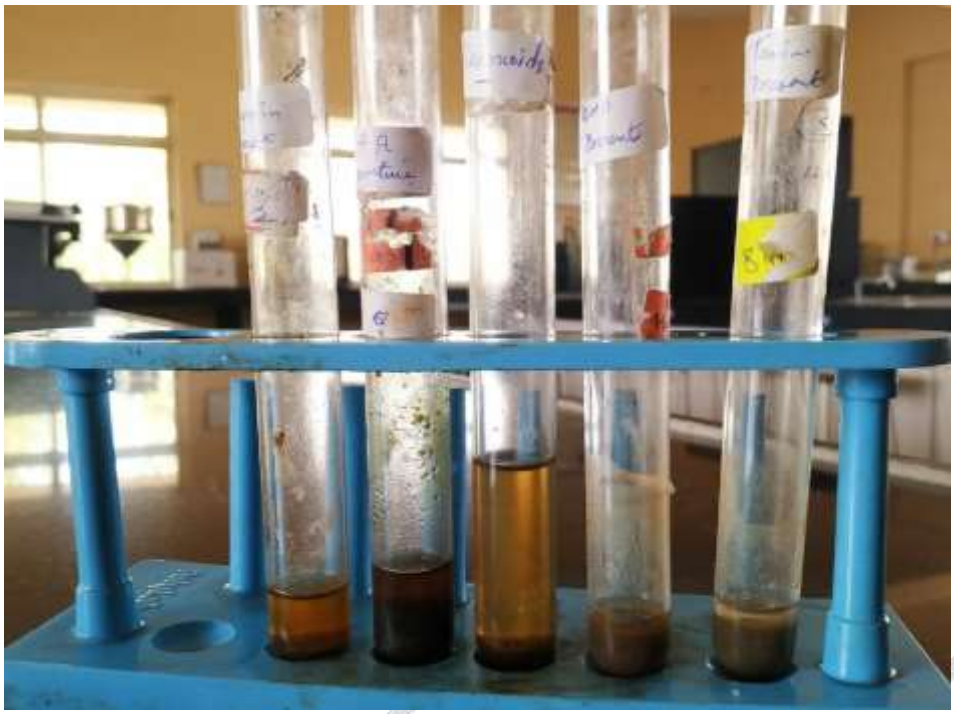

Fig4: Phytochemical analysis of ethanolic extract of Dendrophthoe falcata extract.

1.Test for Alkaloids:

1.Mayers reagent (KI+Hg2Cl2 solution)

2.Dragondorff's reagent (excess of KI + BiNO3 Solution)

4.Wagners reagent (I2+KI solution)

5.Hager's reagent (picric acid)

1. ***Draggendorf's test:*** The extract was treated with few ml of Dragendroff. Orange coloration of the spot indicated the presence of alkaloids.

2. ***Hager's test:*** The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids

3. ***Wagner's test:*** The extract was treated with few ml of Wagner's reagent. The reddish-brown precipitation indicated the presence of alkaloids.

2.Test for Carbohydrates:

1. ***Molish's test:*** The filtrate was subjected to Molisch's test. Formation of reddish-brown ring indicated the presence of carbohydrates.

2. ***Fehling's test:*** Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.

3.Test for Proteins and amino acids:

1. ***Biuret test:*** 1ml extract + biuret reagent observes for blue colour.
2. ***Xanthoproteic test:***1ml extract + 1ml con. H2SO4 cool and add 40% NaOH observe for yellow colour.

4.Test for Steroids:

1. ***Salkowski test:*** Add chloroform in test tube add conc. H2So4 from the side of test tube. Observe for reddish brown ppt.

2. ***Lieberman Burchardt Test:*** To the chloroform solution in the test tube add acetic unhydride mix add 1 ml of conc. H2So4 from side of test tube and allow to stand. Observe for reddish ring at the junction of two layers.

5.Test for Glycosides:

1. ***Keller Killiani test:*** Add 0.4 ml glacial acetic acid and a few drops of 5 % ferric chloride solution to a little of drug extract. Further add 0.5 ml of conc. sulfuric acid.

formation of blue colour in acetic acid layer is observed.

2. ***Legal Test:*** Dissolve pyridine in the drug extract up on add sodium nitroprusside solution to it and made alkaline, pink or red colour is observed.

3. ***Baljet Test:*** To the extract add sodium picrate it shows yellow colour.

6.Test for Saponins: Foam Test: shake the drug extract vigorously with water foam is observed.

7. Test for flavonoids:

1. ***Shinoda Test:*** To 1ml of extract a piece of metallic magnesium was added, followed by addition of 2 drops of hydrochloric acid.

2. ***Tannins:***2ml of each extract in separate test tube were boiled gently for 2 min and allowed to cool 3 drops of ferric chloride solution was added to each extract.

8.Test for steroid/terpenoid

1. ***Liebermann-Burchardt test:*** To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

EXPERIMANTAL

Method

In-vitro Crystal Inhibition

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), both prepared in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 and 37°C. 950 µL of calcium chloride solution mixed with 100 µL of herb extracts at the different concentrations. Crystallization was started by adding 950 µL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The Cystone tablets are used as standard solution. The percentage inhibition calculated as (OD (controlsample)/OD (control))/100

GROWTH ASSAY 20 ml each of 4mMcalcium chloride and 4mMsodium oxalate were added to a 30 ml of solution, containing NaCl (90 mM) buffered with Tris HCl (10 mM) pH 7.2. To this 600 µl of calcium oxalate monohydrate (COM) crystal slurry (1.5 mg/ml acetate buffer) was added. Consumption of oxalate begins immediately after COM slurry addition and was monitored for 600 s by disappearance of absorbance 214 nm. The relative inhibitory activity was calculated as follows: % relative inhibitory activity = ((C-S)/C) × 100 Where „C" is the rate of reduction of free oxalate without any extract. „S" is the rate of reduction of free oxalate with drug extract.

AGGREGATION ASSAY The method used was similar to that described by Hess et al. with some modifications 'Seed 'CaOx monohydrate (com) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L, both solutions were equilibrated to 60^0c in a water bath for 1 h and then cooled to 37^0c overnight. The Crystals were harvested by centrifugation and then evaporated at 37^0c. COM crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and Nacl 0.15mol/L pH 6.5 then experiments were conducted at 37^0c in the presence or absence of plant extract after stopping the stirring. The rate of aggregation was estimated by comparing the slope of turbidity in presence of extract with that obtain in control.

Ir= (1-Turbidity sample/Turbidity control) *100

RESULT AND DISCUSSION

NUCLEATION ASSAY:

It is the initial step of renal stone formation. The table 1 and figure 5 showed the effect of different concentration of Dendropthoe falcata on nucleation of calcium oxalate crystal formation. The increase in the concentration of Dendropthoe falcata showed increase in the inhibition of nucleation.

Maximum inhibition of nucleation was 67.6% observed at concentration of 100µg/ml.

Table-1: Effect of Dendropthoe falcata and Cystone on nucleation of calcium oxalate crystals

Concentration Ug/ml	O.D of Test	%Inhibition Of test	O.D of standard	% Inhibition of standard
20	0.115±0.002	15.1%	0.106±0.002	22.62%
40	0.098±0.005	28.46%	0.087±0.001	36.49%
60	0.084±0.0015	39.5%	0.065±0.002	52.55%
80	0.070±0.0015	48.9%	0.042±0.002	69.34%
100	0.045±0.002	67.6%	0.027±0.002	80.29%
Control	0.137±0.002		0.137±0.002	

Values are mean±S.D of triplicate

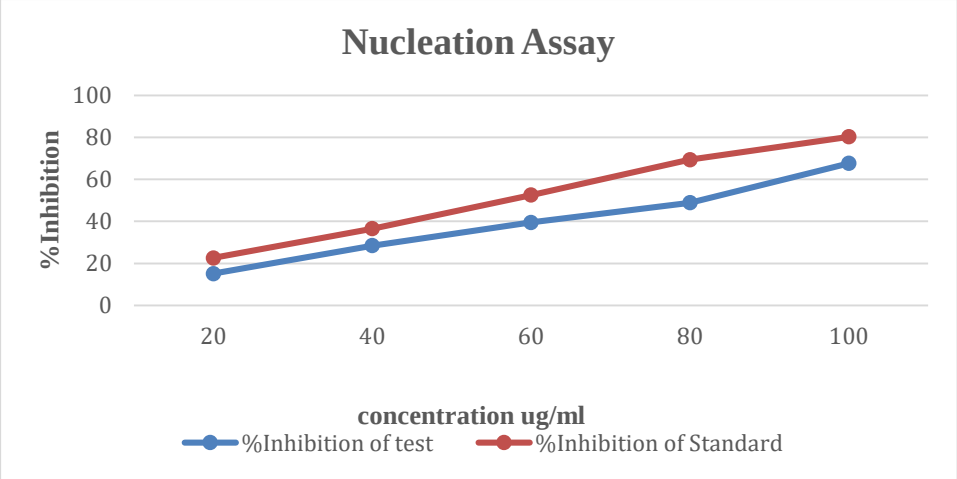

Fig no5: Effect of dendropthoe falcata and cystone on nucleation assay.

GROWTH ASSAY

The table 2 and figure 6 showed the effect of different concentration of Dendropthoe falcata on growth of calcium oxalate crystal formation.

The increase in the concentration of dendropthoe falcata showed increase in the inhibition of growth. Maximum inhibition of nucleation was 66.43%observed at concentration of 100µg/m.

Table-2: Effect of dendropthoe falcata and Cystone on growth assay

Concentration (ug/ml)	O.D of Test	% Inhibition of test	O.D of standard	% Inhibition of standard
20	0.661±0.003	13.02%	0.536±0.02	29.47%
40	0.571±0.001	24.82%	0.454±0.015	40.26%
60	0.444±0.004	41.57%	0.365±0.01	51.9%
80	0.379±0.002	50.31%	0.285±0.015	62.89%
100	0.254±0.002	66.43%	0.202±0.01	73.42%
Control	0.760±0.002		0.760±0.003	

Values are mean±S.D of triplicate

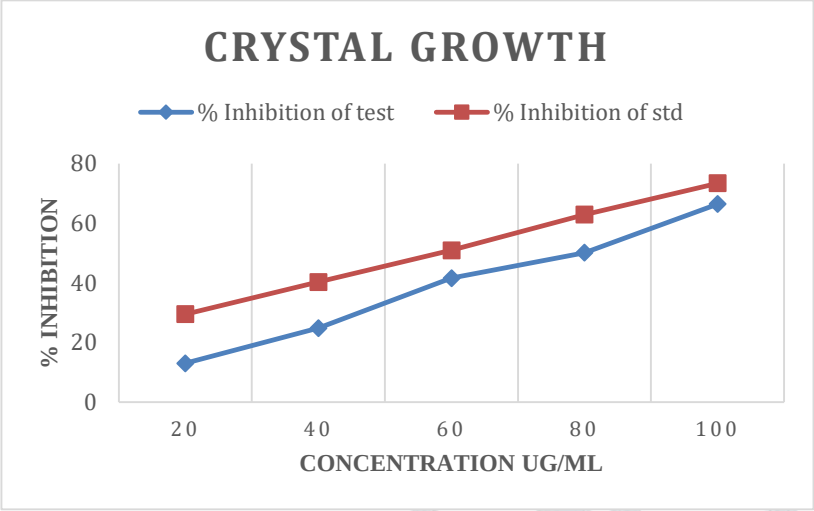

Fig no6: Effect of dendropthoe falcata and cystone on crystal growth.

AGGREGATION ASSAY

The table 4 and figure 7 showed the effect of different concentration of Dendropthoe falcata on aggregation of calcium oxalates. Crystal formation. The increase in the concentration of dendropthoe falcata showed increase in the inhibition of aggregation. Maximum inhibition of aggregation was 40.74%observed at concentration of 100µg/ml.

Table-4: Effect of dendropthoe falcata and Cystone on aggregation assay

Concentration (ug/ml)	O.D of test	% Inhibition of test	O.D of standard	% Inhibition of standard
20	0.385±0.004	19.95%	0.275±0.002	42.82%
40	0.365±0.002	24.11%	0.260±0.001	45.94%
60	0.343±0.003	28.69%	0.215±0.002	55.3%
80	0.310±0.002	35.55%	0.166±0.002	65.45%

100	0.285±0.003	40.74%	0.098±0.003	79.62%
Control	0.481±0.002		0.481±0.002	

Values are mean ±SD of triplicate

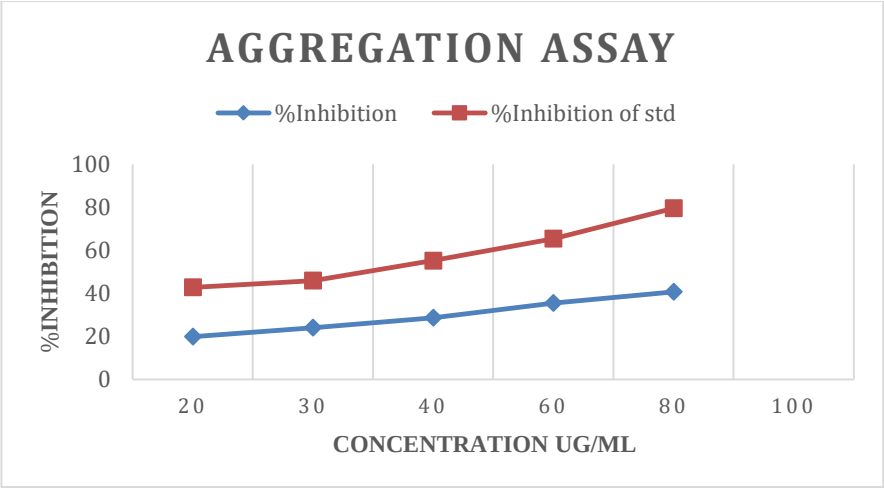

Fig no7: Effect of dendropthoe falcata and cystone on aggregation assay.

The result shows that Dendrpthoe falcata shows significant antiurolithiatic activity, but there is a need of detailed in vivo study to prove the antiurolithiatic activity

Kidney stone are hard solid particles in the urinary tract. In many cases, stones are small and pass out of the body without any problem. If the stone blocks the flow of urine, excruciating pain may result and prompt medical treatment is needed. Kidney stone formation is a complex process of various physicochemical events including urinary supersaturation, nucleation, growth, aggregation and retension of crystals in the renal tubules. Urinary supersaturation generally considered to be the one of the causative factors of callogenesis. Calcium oxalate monohydrate crystals are harmful than calcium oxalate dehydrate crystals because of their tendency to attach with kidney epithelial cells resulting in the form of kidney stone.

Supersaturation of urine followed by cluster formation which leads to the process of nucleation. In this process the phase changes of dissolved salts into solid. Then crystal growth and aggregation. Aggregation is the most effective mechanism to increase the size of the particle, composition and structure of urinary crystals. In the present study help to determine the effect of dendropthoe falcata extract crystal nucleation, growth and aggregation. The result shows that the extract possess significant inhibitory activity in crystal formation, it may be due to presence of various phytochemical such as flavonoids, saponins, terpenoids.

Phytochemical Analysis

The phytochemical analysis of the extract is shown in below table 5. The antiurolithiatic activity of Dendropthoe falcata due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, steroids, tannins etc.

Table-5: Pharmacognostic Screening of the entire plant powder of D. falcata

Sr. No.	Test	Result
1.	Foreign organic matter	-
2.	Ash value a. Total ash b. Acid Insoluble c. Water soluble	 5.5% 1% 1%
3.	Extractable matter a. Water extract b. Alcohol extract	 6.9% 11.5%
4.	LOD	8.7%
5.	Swelling factor	2 ml.
6.	Volatile oil	-
7.	Tannins	-
8.	Foaming Index	117.95
9.	Limit test for Arsenic	Passed
10.	Limit test for Lead	Passed

Table-6: Preliminary phytochemical screening of the entire plant powder of D. falcata

Test	Chloroform	Ethanol	Water
Alkaloids 1. Dragendroff test	+	+	-
2. Wagner's test	+	+	-
3. Hanger's test	+	-	-

Carbohydrates 1. Molish test 2. Fehling's test	- -	- -	+ +
Protein & Amino acid 1. Biuret test 2. Xanthoprotein test 3. Lead acetate test 4. Ninhydrine test	- - - -	- - - -	- - - -
Steroids 1. Libermann Burchard test 2. Salkowski test	- +	- -	+ -
Glycoside 1. Legal test 2. Baljet test 3. Bontrager test 4. Keller Killiani test	+ + - +	- - + +	- - + +
Saponins	+	+	+
Flavonoids 1. Shinoda test	+	+	+
Phenolic compounds and tannins	+	+	+
Triterpenoid	-	-	-
Fixed oil Saponification test	+	+	+

+ Present, - absent.

Presence of various phytochemical such as flavonoids, saponins, terpenoids in the extract show the antiurolithiatic and hence the crystal formation is controlled and helps to reduce its further growth and recurrence.

SUMMARY & CONCLUSION

Summary: Herbal medicine is used by up to 80% population in dendrophthoe falcata is used in traditional medicine and to treat ulcers, asthma, impotency, paralysis, skin diseases, menstrual problems, pulmonary tuberculosis and wounds. The presence of kaempferol, quercetin is noted and its alcoholic extract was found effective. Significant decrease on the renal calculi development was noted. There are many potential effects of diet to increase or decrease the risk of renal damage. Kidney stones have been attributed to excessive dietary intake of calcium, oxalate, protein and salt as well as to diminished intake of fluids. The present

study evaluated the ant urolithic activity of extract of dendrophthoe falcata this work was performed by using in-vitro model. This study give evidence for plant dendropthoe falcata possesses antiurolithiatic activity.

Conclusion: The present study conclusively demonstrates that dendrophthoe falcata a good source of various from phytochemical plant contain chemical constituent like Flavonoid, Pentacyclic triterpenoid, Saponin glycoside Gallic acid, Cardiac glycoside. The plant show in-vitro crystal inhibition action. It might be because of presence of saponin. So, this experiment will be helpful in future for scientific evaluation constituent which show for urolithiasis activity in-vivo.

FUTURE SCOPE

The synthetic drugs used to prevent urolithiasis are not effective in all patients, and many of them have adverse effects that compromise their long-term use. In the present-day management of urolithiasis with open renal surgery is an unusual and rarely used one since the introduction of Extracorporeal Shock Wave Lithotripsy (ESWL) which has almost become the standard procedure for eliminating kidney stones. Besides imposing the high cost, shock waves in therapeutic doses may cause acute renal injury, decrease the renal function and an increase in stone recurrence. Now-a-days, however, herbal medicine has gained much popularity because, herbal medicines are more effective, have less side effects and reduce recurrence rate of stone formation, hence search for antilithiatic drug from natural sources has assumed greater importance and is promising. Herbal medicines have many phytoconstituents which may exert their beneficial effect in kidney stone treatment. Plant extracts contain phytochemicals that inhibit stone formation by inhibiting synthesis and agglomeration of crystals.

Herbal drug therapy has beneficial effect on kidney and stone recurrence is decreased hence can be used as successful drug in the future.

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