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Preliminary Phytochemical Screening and in Vitro Anti-Urolithiatic Activity of Methanol Extract on Leaves of *Artocarpus Heterophyllus* Lam

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Abstract: ***Urolithiasis is still one of the commonest urological disorders, and herbal extracts offer potential preventive benefits through their diuretic, crystallization-inhibiting, lithotropic analgesic, and anti-inflammatory activities. This study aims to assess the methanolic extract of Artocarpus heterophyllus Lam. leaves as a natural remedy against the formation of kidney stones. The presence of preliminary alkaloids, flavonoids, triterpenoids, saponins, tannins, and phenolic compounds- bioactive constituents renowned for their antioxidant, anti-inflammatory, diuretic, and antimicrobial properties- is essential in the fight against urolithiasis. The extract displayed strong anti-urolithiatic effects in various in vitro tests. Using the titrimetric method, it accomplished around 60% dissolution of calcium oxalate crystals, nearly reaching the 80% efficacy of the standard drug cystone. Dose-dependent inhibition was observed in nucleation, with lower crystal size and count seen at higher concentrations through microscopic examination. These results support the traditional use of A. heterophyllus leaves for urinary disorders and underscore their potential as a safer substitute for synthetic treatments. Future in vivo and clinical studies are recommended to elucidate mechanisms, optimize formulations, isolate active compounds and establish safety profiles, paving the way for plant-based therapeutics in renal healthcare.***

Keywords: Artocarpus heterophyllus, urolithiasis, anti-urolithiatic activity, calcium oxalate, titrimetric assay, nucleation inhibition, aggregation assay, phytochemical screening

1. Introduction

Healing herbs have taken pride of place in the therapeutic armoury of human beings from times immemorial. Over 25% of pharmaceuticals in the developed world are derived from plants, directly or indirectly[1]. They are also a panacea to prevent against variety of diseases[2]. The Medicinal plant knowledge has been developing over the centuries upon various systems of medicines like Ayurveda, Unani and Siddha[3]. In a time when synthetic medicines did not exist, people's health relied completely on these plants. Due to the fact that use of synthetic drugs contributes significantly to an increase in the number of adverse drug reactions, people have gone back to nature for safe cures[4]. According to the World Health Organization it is estimated that about 80% of the world's population living in developing countries rely on traditional plant-based medicine for their health care. Medicinal plants are the "backbone" of traditional medicine; more than 3.3 billion people in developing world use these medicinal plants regularly[5]. Chemical principles from herbal traditions are greatly simplified and characterized by new drugs on the basis of medicinal plants[6]. Medicinal plants are attractive sources for both indigenous medicine and trade commodities in response to the demand for new drug discovery from distant markets. In order to better integrate these plants into medical establishments, the professionals must be trained in conventional and traditional application of plant derived compounds[7]. In India, several medicinal plants of the 'Pashanabheda' group are reputed to be beneficial in cases of urinary stones according to the Ayurvedic system of medicine. 'Pashanabheda' is the name used for some plant that has diuretic and anti-urolithiasis properties in Sanskrit[8,9]. Each civilization has its own knowledge and experience of the therapeutic properties of plants. The perception and experiences of the commonly used medicinal plants, raised people's interest for these plants to be used against urolithiasis[10]. The herbal extracts somehow reduce the chances of stone formulation as they may possess various properties such as diuretic action, crystal inhibition activity, lithotrophic activity, pain relieving and inflammation reducing[11].

Urolithiasis has been prevalent among human beings since long time, with serious health and biological problems. It comes from the Greek 'ouron' (urine), 'oros' (fow) and 'lithos' (stone). It is a heterogeneous and multifactorial urologic disease, with formation of calculi in the kidney, bladder or urinary passage[12]. Bladder stones have been discovered in the mummies of ancient Egyptians and in early Native American burial sites dating from 4000 to 1500 BC. The early Sanskrit documents in India (3000-2000 BC) mention stone formation[13].

Renal calculi has become more prevalent in most of the Asian countries in the last few decades including Saudi Arabia (6.8% to 19.1%), Iran (5.7% to 8.1%), Israel (1.2% to 9.2%) South Korea (3.5% to 11.5%) and Thailand (1..4.to16·->: D)., China (from 4% to 6.4%), Japan (from 4.3% to 9.0%) and South Korea (from 3.5% to 11.5%). The prevalence of kidney stones was found to be 5.2 percent among adults 20

to 74 in North America between 1988 and 1994. There was 8.8 percent incidence between 2007 and 2010 throughout this period. The prevalence of urolithiasis was higher in southern US residents than it had been for western or northeastern US residents[14].

Urolithiasis is the condition that corresponds to the presence of calculi (singular calculus) in the urinary tract and encompasses Nephrolithiasis (Renal or Kidney stones), Ureterolithiasis (Ureter stones) and Cystolithiasis. The disease is shown in all young to greater than 70 years. After initial stone, the risk of stone recurrence is 10% at one year, 35% at five years and 50% at ten years[15].

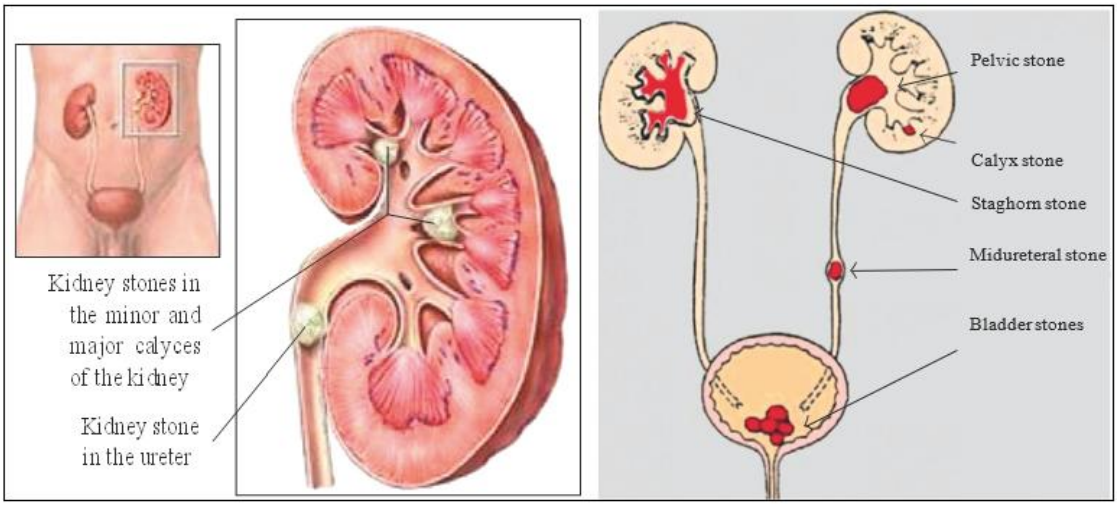

Figure 1: Kidney stone locations in the urinary system[17]

Risk factors:

The etiology is multifactorial with genetic, environmental and lifestyle as risk factors. Sexual dimorphism is well recognized with a male predominance in earlier years, sharing risk factors but now with an emerging female prevalence. Geographically, urolithiasis varies and is more common in industrialized areas and regions with an arid climate[16].

Life-style habits and dietary/nutritional factors

Including high consumption with animal proteins, salt, and inadequate intake of chelating agents (citrate), fiber, alkali foods.

Metabolic

Including hypercalciuria, hypocitraturia, hyperoxaluria, hyperuricosuria and a history of gout (poor uric acid metabolism).

Hypercalcemic disorders

Primary hyperparathyroidism and the other hypercalcemic disorder of calcium metabolism.

Urinary composition

Increased excretion of urinary crystallization promoters and reduced excretion of inhibitors (urine with lack of inhibitory substances).

Low urine volume

Insufficient consumption of water (that leads to dehydration and supersaturated urine).

Repeat urinary tract infections

Abnormal pH of the urine and urinary alkalinization due to bacterial urease (for example, Proteus mirabilis).

Genetic predisposition/inherited disorders

Family member history of stones (genetic susceptibility); genetic monogenic diseases (disorders of a single gene on autosomes); renal tubular acidosis.

Anatomical defects

Congenital diseases such as medullary sponge kidney, ureteropelvic junction stenosis, pyeloureteral duplication and polycystic renal disease or Horseshoe kidney.

Lithogenic Drugs

Indinavir (protease inhibitor) and sulfonamides (sulfadiazine), uricosuric agents with low solubility favoring calculi formation, long-acting ceftriaxone.

Hypertension, Obesity, Climate Warming (Global warming), occupation, geographic locations, seasonal changes (summer> Winter), Inflammatory bowel disease and other enteral malabsorption or Conditions Absence of intestinal oxalate- degrading bacteria [17].

Types of kidneys stones:

Urolithiasis is divided into four types and nine subtypes according to composition and etiology: non-infection stones (calcium oxalate, calcium phosphate, uric acid), infection stones (magnesium ammonium phosphate, carbonate apatite, ammonium urate), genetic factors (cystine, xanthine, 2,8-dihydroxyadenine), and drug-related stones [18]. The chemical character and the shape and size of kidney stones result from urine abnormalities. They are generally divided into five types according to mineral constituents and pathogenesis.

Calcium stones

Such as calcium oxalate and calcium phosphate. Calcium stones - calcium combined with either oxalate, phosphate or carbonate in the urine that then crystallizes. Of all renal (kidney) stones, calcium makes up the greater majority-approximately 80%. The proportion of calcium stones might

be Apatite (5%) and a mixture of the two (45%). The primary component is calcium. The majority of kidney stones are made of either CaOx monohydrate (COM, whewellite or $Coco_4 \cdot H_2O$) or (COD, weddellite or $^2CaC_2O_4 \cdot 2H_2O$), whereas more than 60% of the stones contain both types of calcium oxalate. COM is the type of stone with high thermal stability. COM is more common in clinical stones than COD. Recurrence is further considered to be higher when compared to other types of kidney stones[17].

Uric Acid Stones

About 3-10% of kidney stones are made up of uric acid. Diet rich in purine, especially animal protein, would also cause hyperuricosuria, low urine volume and decrease of pH of urine (<5.05), which is a risk factor to form uric acid stone[17]. Gouty arthritis patients can form kidney stones. The most common cause of uric acid nephrolithiasis is idiopathic[19]. Changes in diet, such as decreasing salt consumption and reducing animal protein, are also beneficial in prevention of uric acid stones[20].

Struvite (magnesium ammonium phosphate) stones

Also known as the infection or triple phosphate stones make up 10–15% of kidney stones[21]. Form in the urinary tract as a result of infection with urease-producing bacteria such as Klebsiella or Proteus[22].Struvite stones can spontaneously pass if they are small, but large stones need to be treated [23].

Cystine stones

These make up less than 2% of all types[17]. Cystine stones result from the precipitation of cystine, a substance which is poorly soluble in urine and contains sulfur[24]. Cystine stones (due to cystinuria, an inherited disorder), result from high urine concentration of cystine which subsequently crystallizes in the kidneys, ureters or bladder[25]. They may need conservative treatment for pain and operative intervention as in cystitis calculi[26]. Constitutes ~1% of kidney stones; associated with sulfa drugs, triamterene, atazanavir, guaifenesin and indinavir (used for HIV treatment). They developed by deposition of drug/metabolite stones or may result from disruption of the purine or calcium oxalate metabolic pathways[17].

Mechanism of Stone Formation

Biomineralization of kidney stones is a biochemical event that occurs as result of supersaturation, resulting in solute precipitation, nucleation and subsequent crystal growth. It varies with ion strength, pH and substances, such as calcium oxalate, or uric acid. The risk is increased with low urine volume and the mechanism includes thermodynamics (nucleation) as well as kinetics (crystal growth). This stone is formed due to imbalance of urine inhibitors and promoters. The main modes are described as nucleation, growth aggregation and crystal holding. Prevention aims to avoid supersaturation[17].

Supersaturation of urine

Urinary supersaturation is when the solubility limit of excreted (calcium, oxalate and phosphate) substances is exceeded in urinary content; hence it results to crystals. The excessive solutes come together to become small crystals, which then join together as large crystals. Such factors as supersaturation, pH and inhibitors can have a major impact with regard to the formation of crystals as well as their growth which results in kidney stones. The knowledge of these parameters is essential for prevention and therapy[28].

Crystallization

Calcium oxalate (CaOx), calcium phosphate, and uric acid are common components of kidney stones because they exist at a high concentration in urine[29]. When urine becomes oversaturated, crystals form and some of them have characteristic features. They can further predispose to stone formation by causing obstruction to urine flow and crystallization of the urine[17]. Urine pH, mineral concentration and inhibitors influence crystal formation. Knowledge of the processes involved in crystal formation and growth is central to devising successful strategies for the prevention and treatment of kidney stone disease[30].

Crystal Nucleation

Renal calculi form by supersaturation of the urine and particles coalesce to precipitate either with free or fixed nucleation on cells, casts, RBCs or crystals. Mucopolysaccharides and nanobacteria facilitate this process, thus nucleation is a possible major target in prevention[17]. There are two primary types of nucleation: homogeneous, which takes place within solution, and heterogeneous, which can take place over a foreign particle or solid surface[28].

Growth of crystals

Crystals develop from formed nuclei through the entry of ions or molecules into the crystal lattice and grow at a rate dependent on urine salt concentration, pH, as well as inhibitors and promoters. If the salt concentration is high, there is supersaturation, and crystal growth is promoted. Some stone types, such as struvite, are facilitated by acidic urine and even urine saturated with crystalloids due to enhanced surface charge of particles leading to favorable contact of ions. Therefore, inhibitors and promoters are important for crystal or stone formation[31,32].

Crystal aggregation

Crystal aggregation, the phenomenon of uniting small crystals within urine into larger particles, is especially important in calcium oxalate (CaOx) urolithiasis. Although nucleation and growth are important process in the formation of CaOx stone, aggregation plays a key role by allowing retention of crystals within the renal tubules or urinary tract and is considered the critical step between initial crystal formation to clinically relevant stones[33].

Crystal cell interaction

Crystallization proceeds by addition of ions to the lattice having formed the nuclei, and is determined by factors, such as salt concentration, urine pH and presence or absence of growth- inhibitors or promotion substances. Under high salt concentration and supersaturation, stone enlargement is accelerated, whereas pH changes may dictate types of stones. Inhibitors such as citrate and magnesium, attach to crystal surfaces and hinder their growth promoting substances, i.e. calcium and oxalate improve crystal growth by increasing surface charge which facilitates addition of more ions [28].

Table 1: Represents kidney stone inhibitors and their mechanisms[34]

Name of the inhibitor	Mechanism
Citrate	Citrate can inhibit calcium phosphate crystallization by chelating calcium ions, decreasing CaOx level and preventing crystal nucleation and aggregation. It induces Tamm-Horsfall protein (THP) activity, which cannot only inhibit the aggregation of CaOx crystals but it also inhibits osteopontin (OPN), an important protein in urinary calculi. Furthermore, citrate will increase the pH of urine and calcium-phosphate complexes that would be less likely to deposit or crystallize due to alkaline urine.
Magnesium	Magnesium Chelate: Oxalate Absorbance + Excretion Magnesium decreases oxalate absorbance and excretion in the GI. Urine Citrate In magnesium-deficient persons, dietary or oral sources of magnesium increase urine citrate.
Inter-alpha-trypsin inhibitor family of proteins	Inter-alpha-trypsin inhibitor (IαI) In tubules, the Kunitz-type protein IαI has been described to possibly inhibit CaOx crystal adhesion 72. In studies, it was shown that IαI inhibits CaOx crystallization and protein fragments of IαI were detected in urine suggesting an involvement in the formation of CaOx stones.
Phytate	Phytate also diminishes the formation of calcium stones by capturing calcium in the GI tract, thereby forestalling absorption, and discouraging kidney and urinary system stone- creation.
Potassium	Potassium lowers the risk of kidney stones by reducing urinary calcium excretion, which decreases the level of urine to crystalline calcium.
Pyrophosphate	Pyrophosphate and diphosphate inhibit calcium phosphate precipitation and stone formation, while pyrophosphate suppresses calcium absorption by stimulating 1,24,25-trihydroxy vitamin D production.
Tamm-Horsfall protein (THP) or mucoprotein	Uromucoid (uromodulin) inhibits COM crystals aggregation in the urine, particularly at high pH, low ionic strength, and/or diminished levels of divalent ions and THP. Its efficacy is pH, calcium, sodium, hydrogen ions and THP dependent.
Osteopontin (Uropontin)	Osteopontin (OPN) inhibits CaOx crystal growth by preventing the crystals from associating with epithelial cells, decreasing their adhesion over urinary tract surfaces and in turn likely reducing kidney stone formation.
Glycosaminoglycans	Glycosaminoglycans (GAGs) such as chondroitin sulfate, heparin sulfate, and hyaluronic acid in urine are potent inhibitors of CaOx crystallization, growth, and aggregation.

Table 2: Represents kidney stone promoters and their mechanisms[34]

Name of the promoter	Mechanism
Dehydration	Concentrated urine is more likely to promote urinary stone formation.
High dietary intake of oxalate	A diet high in oxalate results in increased urinary oxalates with the potential of forming CaOx crystal or stones.
High animal protein dietary intake	Urine calcium can be enhanced by animal protein intake which may predispose to urinary stones.
Family history of urolithiasis	Urolithiasis has a hereditary component and stone risk rises in the presence of a positive family history.
Hyperparathyroidism	Hyperparathyroidism causes increased calcium excretion into the urine, which increases the likelihood of developing a urinary stone.
Cystinuria	Cystinuria is a genetic condition which results in cystine stones due to defective reabsorption of cystine.
Obesity	Obesity also elevates urinary calcium and negatively changes metabolism, which can lead to the development of stones.
Metabolic syndrome	Metabolic syndrome involving obesity, insulin-resistant diabetes hyperlipidaemia enhances stone formation by altering urine chemistry and inflammation.
List of certain drugs	Urinary stone risk can be modified by medication exposure, such as use of diuretics, calcium antacids and protease inhibitors.
Urinary tract infection	UTIs increase risk for kidney stones by changing the urine manufacturing, pH and the encouragement mineralisation formations and aggregations.
Chronic kidney disease	CKD increase the risk of kidney stone of modifying the composition of urine and obstructing waste elimination.
Age	Due to changes in the composition of urine and greater probability for comorbidities, aging predisposes men to kidney stones.
Gender	Men are affected with Urolithiasis more frequently that women because of urinary anatomy and hormones.
Climate	Hot, dry climates increase stone risk through evaporative losses of fluids and ions. And reducing urine output.

2. Materials and Methods

Extraction of plants:

The methanolic extract of *Artocarpus heterophyllus* was obtained by maceration. Whole leaves of A. heterophyllus, freshly arrived from the farm, were harvested and washed thoroughly to get rid of any surface pollutants. The leaves were shade-dried at room temperature according to standard methods used for plant product preparation in order to conserve heat sensitive phytochemicals. After drying completely, leaves were finely powdered with a sterilized pestle and sieved through NO.85 mesh sieve to make homogenous particle size. For extraction, Preparation of extract 30 grams of powdered leaf sample was macerated with 250 ml of methanol at room temperature for 72 h. The solvent penetration and the yield of phytochemicals were maximized by continuous shaking on an orbital shaker for occasionally. The mixture was filtered after extraction to eliminate any insoluble plant debris. The filtrate was evaporated on a water bath at 30–40°C, gentle heat was used to avoid decomposition of thermolabile compounds; the solvent was completely evaporation. The concentrated extract was kept in desiccator to avoid moisture absorption and stored until further phytochemical and bioactivity studies were carried out[63][64].

FTIR:

Fourier transform infrared spectroscopy (FTIR) is the most

useful and powerful analytic tool for the determination of functional groups in chemical compounds. It accesses vibrational transitions of chemical bonds by recording the absorption of specific IR radiation wavelengths, all characteristic for certain types of bonds: hydroxyl, carbonyl- and aromatic groups. This enables the characterization of molecular structure in great detail. FTIR is particularly suitable for the analysis of complex mixtures such as plant extracts, which consist of numerous bioactive components like phenolics, flavonoids, alkaloids, and carbohydrates. Using the IR spectrum an approximately idea of possible presence compounds and classes of groups contributing to these extracts can be identified giving a significant indication about extract chemical content and potential biological activity. The methanolic extract of *Artocarpus heterophyllus in* the present work was dried as solid and reduced to fine powder. It was mixed with KBr (spectroscopic grade) in a ratio of about 1:100 under pressure and formed into the transparent pellet using a hydraulic press. The pellet was inserted in the sample holder of FTIR spectrophotometer (Shimadzu, IRAffinity-1, Japan). The spectra were measured in mid-infrared (MIR) 4000–400 cm^{-1} wavenumber region with a resolution of 4 cm^{-1}. A background scan was carried out with a pure KBr pellet before sample measurement in order to eliminate atmospheric and instrument interferences. Spectra obtained were analyzed to spot characteristic absorption bands of the functional groups attributed to methanolic extract of Artocarpus heterophyllus.

In-vitro anti-urolithiatic activity

Titrimetric assay method

This technique was used to assess the potential of plant extracts in dissolving established kidney stones. The process steps are as follows:

Step 1: Preparation of experimental kidney stones (Calcium oxalate stones) through homogenous precipitation

In a beaker, adequate quantity of distilled water containing stoichiometric proportion of calcium chloride dihydrate and sodium oxalate in 10 ml of 2N H2SO4 was mixed. The precipitate was calcium oxalate. The precipitate was washed with ammonia solution free from sulphury acid. The filtrate was then washed with deionized water and dried at 60°C for 4 h.

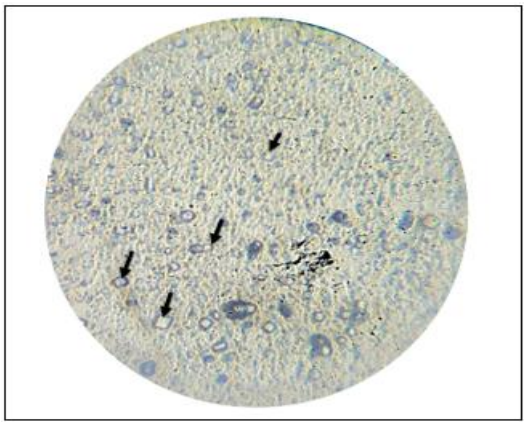

Figure 2: Calcium oxalate crystals formed under a compound microscope (at 10x magnification)

Step 2: Preparation of the semi permeable membrane from eggs

This membrane is sandwiched between the outer calcified shell and the inner material including albumen and yolk. The shell was dissolved chemically by incubating the eggs in 2 M HCl for 24 h, resulting in complete decalcification. In addition to this, wash with distilled water and very carefully using a sharp pointer make a hole at the top and remove all content from decalcified egg. The same was washed well with steam-distilled water, kept in the condition of moisten and were loaded into ammonia solution and rinsed off using steam distilled water. Last, it is refrigerated at a pH of 7–7.4[68]. A 500 mg Cystone tablet in absolute ethanol for discoloration removal yielded 400 mg. The tablet was powdered and cystone was dissolved in distilled water (100 ml) and filtered. The positive control for in vitro anti-urolithiatic activity was the filtrate of Cystone. 0.32gm of KMnO4 was dissolved in 100ml distilled water to obtain 0.02M KMnO4 solution. It was boiled for 30min. Following cooling, excess of MnO4 was filtered off [69].

Figure 3: Eggs kept in 2M HCl for 24 hours for decalcification

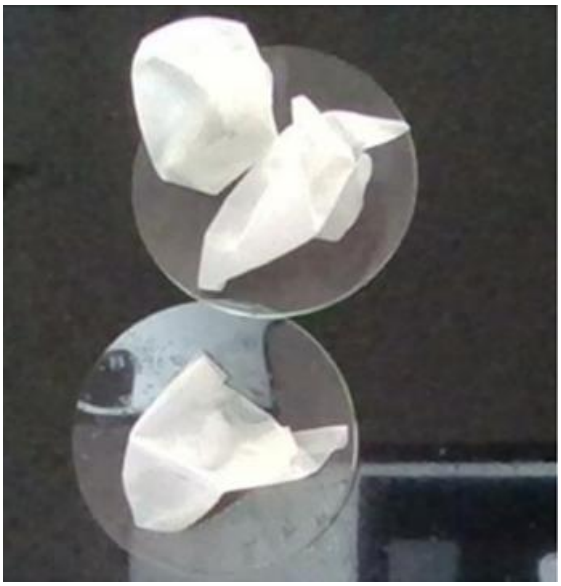

Figure 4: Semipermeable egg membrane

Step 3: Estimation of Calcium oxalate by Titrimetric

The dissolution percentage of calcium oxalate was determined by weighing exactly 10 mg of the experimental calcium oxalate and 10 mg of seed extract which were packed in one-and-half-glass setup using a semipermeable membrane. The semipermeable membranes were pre-weighed. It was allowed to settle down in a conical flask with 100ml 0.1 M Tris buffer (Figure 5). There were two groups, the first as blank with only 10mg of calcium oxalate. Positive control: In this group 10 mg of calcium oxalate was given along with standard drug cystone10mg. The third group were administered 10mg of calcium oxalate + methanol extract of Artocarpus heterophyllus. All the conical flasks of the groups were incubated in an incubator for 7-8 hours. Semi-permeable membranes from each group were decanted in to individual test tubes. 2 ml 1N sulphuric acid was added to each test tube and titrated with 0.9494 N KMnO4 till a faint pink color end point was attained[70].

% Dissolved of calcium = [(C-T)/c] x 100

Where, C= There remaining precipitate of CaOx in control (mg),

T= amount of precipitate of CaOx after the test solution was employed (mg).

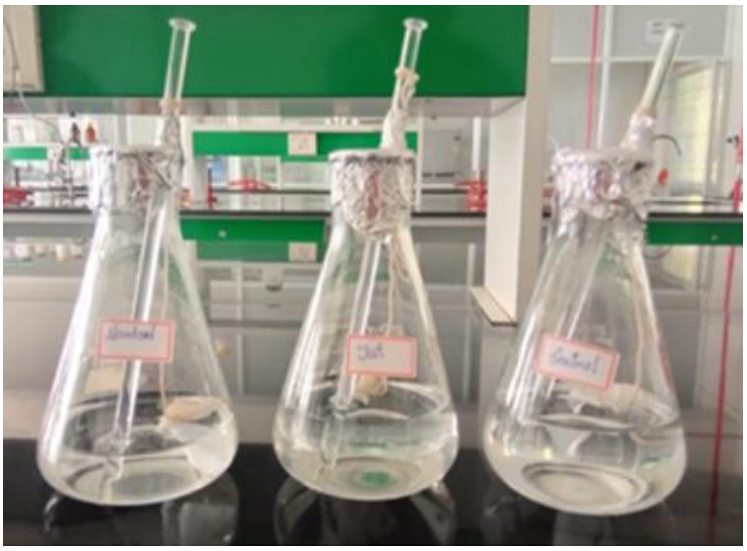

Figure 5: Calcium oxalate and inhibiting extracts enclosed in semipermeable and suspended in Tri's buffer

Nucleation assay method

The nucleation assay is based on the crystal dissolution absorbance increases with the increase in the extent of crystal dissolution and can be measured spectrophotometrically at (620nm.) Nucleation experiment was performed to investigate the influence of methanolic extract from *Artocarpus heterophyllus* on calcium oxalate (CaOx) crystallization. Calcium chloride (CaCl2) 5 mmol/l and sodium oxalate (Na2C2O4)7.5 mmol/l solution was prepared in Tris-HCL_0.05 mol/l, NaCl 0.15 mol/l buffer of pH 6.5. Preparation of test sample Fruit pericarp was roentgen dried, powdered to a mesh size and the methanolic extract of Artocarpus heterophyllus dilutions (100 1 by standard method13 in a distilled water from 1000g/ml were prepared. To 1 mL of each concentration methanolic extracts of *Artocarpus heterophyllus were* added 3 ml of CaCl2 solution and three milli litters of Na2C2O4 solution. The ultimate mixes were incubated at 37o C for 30 min. Optical density of these reactions was measured at a wavelength of 620 nm, The experiment was performed in triplicate. Nucleation inhibition percentage by methanolic extract of *Artocarpus heterophyllus* was calculated according to the formula below and compared with that by cystone after consideration as standard concentration [71].

% Inhibition = (1 - OD TEST / OD CONTROL) x100

Aggregation assay method

The aggregation assay is crystal solubilization percent as turbidity rises due to the increase of crystal dissolution determined at 620 nm. Computer calculations of the monohydrate content of experimental calcium oxalate (CaOx) kidney stones were obtained using the procedure described by. Calcium oxalate monohydrate crystals were formed by the mixing of equal concentrations of calcium chloride (50 mmol/L) and sodium oxalate (50mmol/L). Each solution was equilibrated in a water bath for 1 h at 60 °C for the precipitation of Calcium oxalate monohydrate crystals. Crystals were then cooled to 37 °C before air-drying. The CaOx monohydrate crystals were generated at a final concentration of 0.8 mg/ml in Tri's buffer pH 6.5. Then the various concentration of Methanolic extract of *Artocarpus heterophyllus* Lam Samples (100, 200, 600, 800 &1000 µg/ml) were added to the solution of Calcium oxalate monohydrate crystals and incubated at 37 °C for 24 hrs. Cystone (100, 200, 600, 800 and 1000 µg/ml) were employed standard. Turbidity that represents the aggregation activity was calculated based on UV spectrophotometric measured at 620 nm in the presence of the extract and control. The experiment was repeated three times. Average of O.D. triplicates is determined. The average Value is used to calculate. Percentage inhibition and noted down[72].

And the percents inhibition of plant extract was calculated according to

% inhibition = [OD control – OD test] / OD control x 100.

3. Result and Discussion

Preliminary phytochemical test result:
The methanolic extracts of *Artocarpus heterophyllus* Lam. screen indicates the presence of alkaloids, flavonoids, phenolics, tannins, triterpenes and saponins. The Table 3. composites those findings of results.

Table 3: Preliminary Phytochemical screening of methanolic extract of *Artocarpus heterophyllus Lam*

S. No.	Constituents	Test	Result
1	Alkaloids	Dragendoff's Test	+
		Mayer's Test	-
		Hager's Test	+
		Wagner Test	+
2	Flavonoids	Alkaline Reagent Test	+
		Lead Acetate Test	+
3	Triterpenoids	Salkowsi Test	
4	Saponins	Foam Test	+
5	Tannins	Braymer's Test	+
6	Phenols	Ferric Chloride Test	+
		Lead Acetate Test	+

FTIR

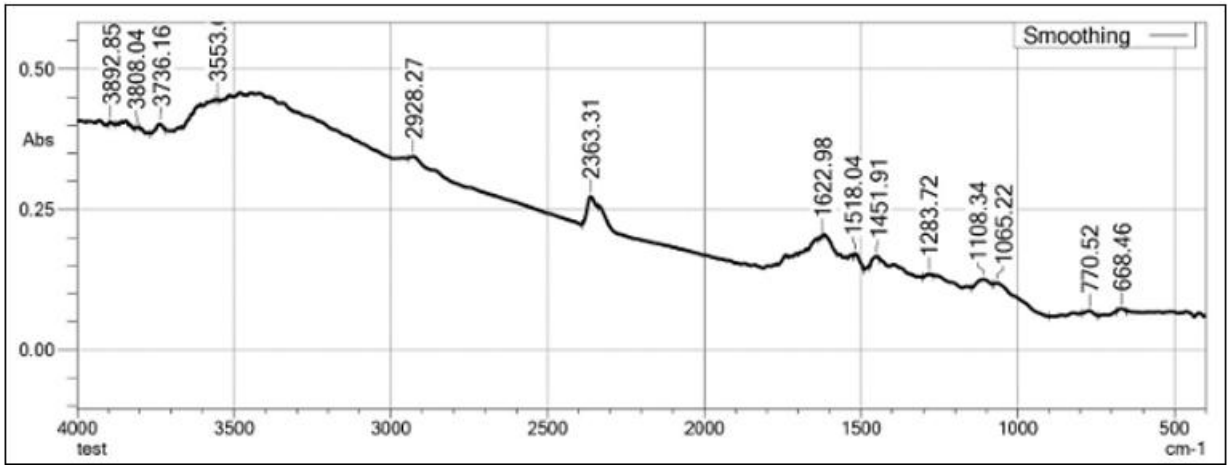

Figure 6: FTIR of Methanolic extract of *Artocarpus heterophyllus Lam*

Discussion
The FTIR spectrum of the methanolic extract of *Artocarpus heterophyllus* Lam. leaves exhibited distinct peaks, suggesting the presence of various bioactive phytochemicals. A large absorption peak at 3275–3400 cm^{-1} might be attributable to the presence of hydroxyl (-OH) groups, which indicated the presence of phenolics, flavonoids, alcohols and saponins. The bands 2928–2930 cm^{-1} were assigned to C–H stretching of=CH in aliphatic groups typical for triterpenoids and hydrocarbon, whereas absorptions centered at around 1620–1630cm^{-1} indicated carbonyl (C=O) stretching characteristic of flavonoids, phenolic acids and tannins. Significant bands at 1500–1450cm^{-1} were due to aromatic C=C stretching, indicating the presence of flavonoid and phenolic structures; peaks at approximately 1050–1100 cm^{-1} indicated carbohydrate anomeric C–O stretch for alcohols, ethers, glycosides, phenolics and saponins. The bands centered at around 2928–2930cm^{-1} were due to stretching vibrations of C–H from the aliphatic groups, which are common in triterpenoids and hydrocarbons, and those near 1620–1630cm^{-1} originated from carbonyl (C=O) stretching, typical for flavonoids phenolic acids and tannins. Sharp peaks between 1500 and 1450cm^{-1}representing the stretching forces of aromatic C=C confirmed the existence of flavonoid structures, as well as phenolic structure which was observed as bands appear at their same position; bands close to 1050–1100cm^{-1} corresponded to C–O stretching peculiar to hulls, ethers, glycosides, phenolics and saponins. Furthermore, lower wave number signals at 670-700 cm^{-1} were attributed to aromatic ring bending, providing further evidence of the occurrence of flavonoids and tannins. Taken together, these spectral features are in close agreement with results of the preliminary phytochemical screening, indicating the presence of alkaloids, flavonoids, phenolic compounds tannins triterpenoids and saponins. The FTIR results therefore afford direct proof of functional groups associated with antioxidant, anti-inflammatory, and antimicrobial activities in the extract[73].

Titrimetric method:

Table 4: Calcium Oxalate Dissolution by Test and Standard Drug

Group	Volume of Standard KMnO4	Wt. of Calcium Estimated	Wt. of Calcium Reduced	% Dissolution
Control	1 ml	3.65	0	0
Standard (Cystone)	0.2 ml	0.73	2.92	80%
Test Drug	0.4 ml	1.46	2.19	60%

Discussion

Titrimetric assay The percentage dissolution of calcium oxalate crystals by the reference drug Cystone and test drug was found to be pronounced when compared with untreated control. The control had the highest retention of calcium oxalate (3.65 mg), which was set as the baseline. The first was Cystone, which had 80% solution rate, in accordance with the known performance of this product to treat urolithiasis. The test drug exhibited good activity of 60% dissolution, the results of which are indicative of a positive antiurolithiatic potential. The dissolution is attributed to the redox of potassium permanganate and residual calcium oxalate, which results in a smaller amount of titrant used for larger crystal dissolution. This assay is an accurate end point for anti-crystallization activity The dose-response relationship confirms the clinical efficacy of the tested substance which nearly equals that of Cystone. Conclusively, KMnO4 titrimetric assay is still an effective and widely employed tool for anti-urolithiatic activity screening.

Nucleation assay:

Table 5: Measurement of UV Absorbance value of the methanolic extract of *Artocarpus heterophyllus* Lam. with standard drug

Treated Sample	Optical Density			Mean ± SD
	Replicate 1	Replicate 2	Replicate 3	
Control	0.812	0.792	0.778	0.794 ± 0.017
Test				
100 µg/ml	0.718	0.719	0.717	0.718 ± 0.001
200 µg/ml	0.582	0.581	0.578	0.580 ± 0.002
600 µg/ml	0.491	0.493	0.493	0.492 ± 0.001
800 µg/ml	0.474	0.475	0.476	0.475 ± 0.001
1000 µg/ml	0.259	0.256	0.260	0.258 ± 0.002
Cystone				
100 µg/ml	0.593	0.570	0.569	0.577 ± 0.013
200 µg/ml	0.492	0.495	0.494	0.493 ± 0.001
600 µg/ml	0.286	0.283	0.284	0.284 ± 0.001
800 µg/ml	0.162	0.163	0.163	0.162 ± 0.0005
1000 µg/ml	0.092	0.094	0.095	0.093 ± 0.001

All values are expressed as mean ± SDM (n = 3)

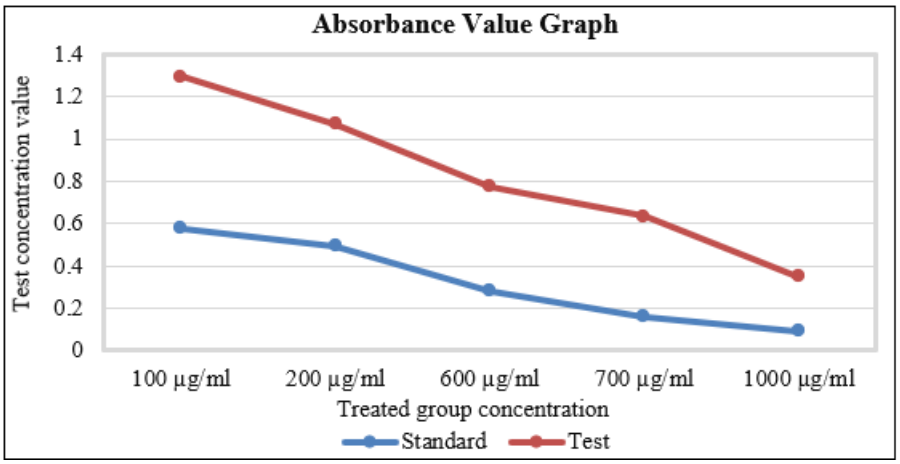

Figure 7: Shows the Absorbance Value Graph of Methanolic Extract of *Artocarpus heterophyllus* Lam. with Standard Drug Cystone

Discussion

The nucleation assay showed a concentration-dependent inhibitory effect on calcium oxalate crystal formation by both the test extract and Cystone. The control was characterized by the highest mean absorbance (0.794±0.017), indicating the highest nucleation activity. The absorption measured gradually decreased with the increasing concentration of test extract from 0.718±0.001 at 100 µg/ml to 0.258±0.002 at 1000 µg/ml. Cystone showed greater inhibition (decreased from 0.577±0.013 at 100-1000 µg/ml to 0.903±0.01) over this range as compared with decreases in ciprofloxacin absorption levels. The small standard deviation at all time points indicates the reproducibility and reliability of the assay. This inhibition, responsive to dose, is in accordance with comprehensive high-impact publications, underlining the nucleation assay centrality for antiurolithiatic potential evaluation and endorsing the translational value of both compounds tested here against kidney stone formation.

Table 6: Percentage Inhibition Value of Methanolic Extract of *Artocarpus heterophyllus Lam.* with Standard Drug Cystone

Treated Sample	% Inhibition
Control	0 %
Test	
100 µg/ml	9.57%
200 µg/ml	26.95%
600 µg/ml	38.04%
800 µg/ml	40.20%
1000 µg/ml	67.53%
Cystone	
100 µg/ml	27.32%
200 µg/ml	37.92%
600 µg/ml	64.25%
800 µg/ml	79.60%
1000 µg/ml	88.29%

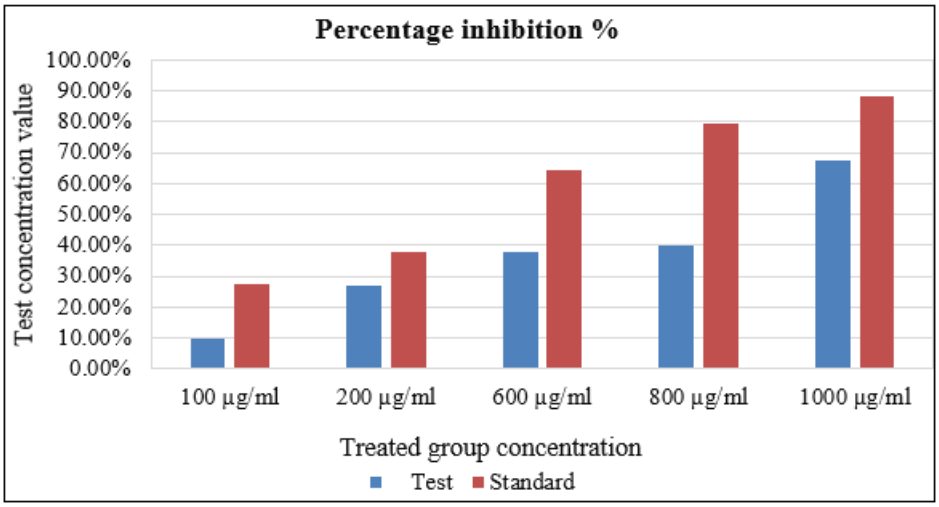

Figure 8: Percentage Inhibition Value of Methanolic extract of *Artocarpus heterophyllus Lam.* with Standard Drug Cystone

Discussion

The nucleation studies reveal that obstruction of calcium oxalate crystallization by the test extract and Cystone is concentration-dependent, thus validating their antiurolithiatic activity. The control showed no inhibitory activity and acted as a negative control. The percentage of inhibition by the test extract increased gradually from 9.57 at 100 µg/ml to 67.53% at 1000 µg/ml whereas, Cystone produced higher inhibition values in all concentrations tested (27.32-88.29%). This dose responsive inhibition confirms both agents' efficacy of the attenuation in the early nucleation phase, a critical step in kidney stone formation. Consistency of percent inhibition with absorbance reduction supports the robustness of the nucleation assay as an in vitro screening platform. All these data confirm the therapeutic potential of the test extract and Cystone, and deserve further pharmacological and clinical exploration for management of urolithiasis.

Aggregation assay method:

Table 7: Measurement of UV Absorbance value of the methanolic extract of *Artocarpus heterophyllus Lam.* with standard drug

Treated Sample	Optical Density			Mean ± SD
	Replicate 1	Replicate 2	Replicate 3	
Control	0.278	0.274	0.274	**0.274 ±0.004**
Test				
100 µg/ml	0.254	0.235	0.273	**0.254 ± 0.019**
200 µg/ml	0.207	0.200	0.180	**0.195 ± 0.014**
600 µg/ml	0.164	0.122	0.191	**0.159 ± 0.034**
800 µg/ml	0.119	0.105	0.186	**0.136 ± 0.043**
1000 µg/ml	0.093	0.097	0.098	**0.096 ± 0.002**
Cystone				
100 µg/ml	0.234	0.235	0.239	**0.236 ± 0.002**
200 µg/ml	0.182	0.180	0.185	**0.182 ± 0.001**
600 µg/ml	0.124	0.127	0.125	**0.126 ± 0.001**
800 µg/ml	0.058	0.056	0.056	**0.056 ± 0.001**
1000 µg/ml	0.032	0.032	0.033	**0.32±0.0005**

All values are expressed as mean ± SD (n = 3)

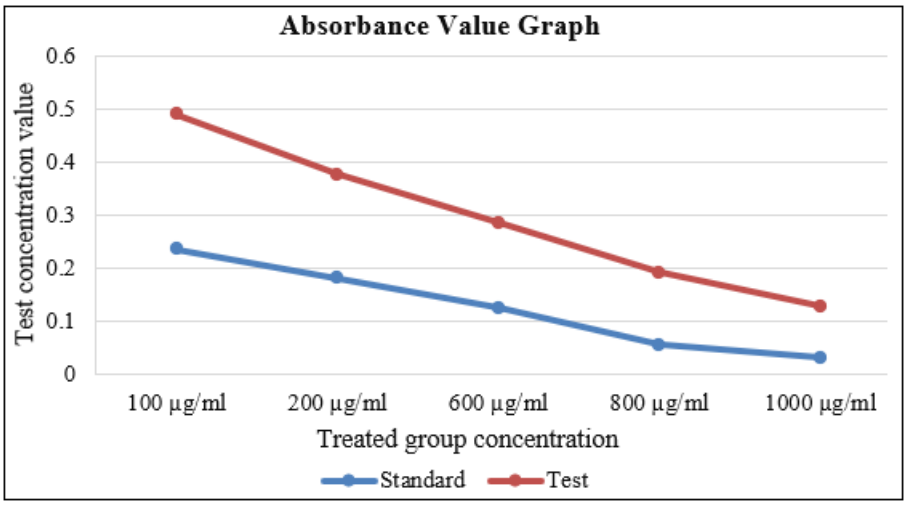

Figure 9: Shows the Absorbance Value Graph of Methanolic Extract of *Artocarpus heterophyllus Lam.* with Standard Drug Cystone

Discussion

In vitro aggregation assay results indicate that the test sample and Cystone (standard drug) prevent particles from aggregating in a dose-dependent manner, as can be seen by decrease in optical density values with increasing concentration (100-1000 µg/ml). All standard deviations are low, with a somewhat higher variation for the test sample at intermediate concentrations, suggesting good consistency of results. The control group continues to have the highest OD, confirming a maximum aggregation in absence of treatment. These results indicate that the anti-aggregatory potential of both compounds is shared between them, but Cystone is markedly more effective and justifies its clinical use and Translate: The test compound has potential for further development as an anti-urolithic or as an anti-crystallization agent.

Table 8: Shows the Percentage Inhibition Value of Plant Extract of EEGG with Standard Drug Cystone

Treated Sample	% Inhibition
Control	0 %
Test	
100 µg/ml	7.30%
200 µg/m	28.83%
600 µg/ml	42.34%
800 µg/ml	50.36%
1000 µg/ml	64.96%
Cystone	
100 µg/ml	13.87%
200 µg/ml	33.58%
600 µg/ml	54.01%
800 µg/ml	79.56%
1000 µg/ml	88.32%

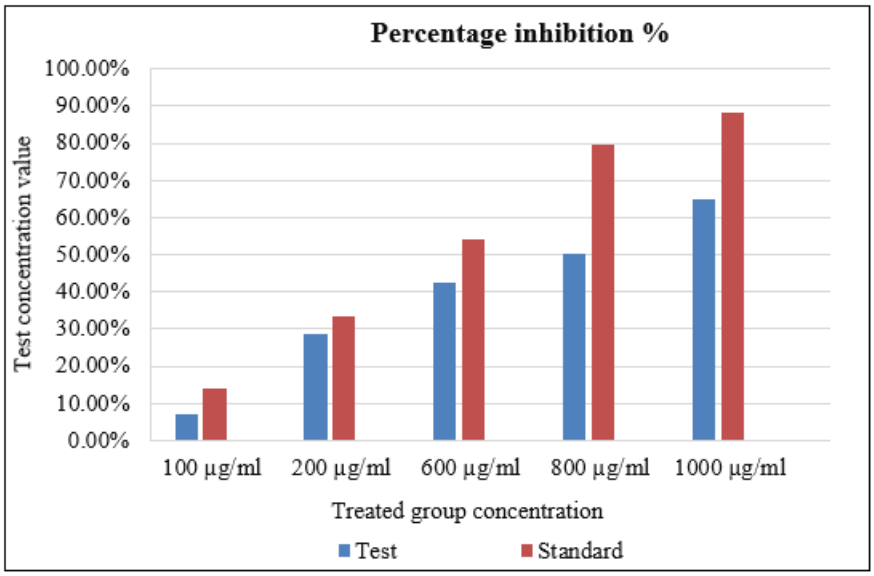

Figure 10: Percentage Inhibition Value of Methanolic extract of *Artocarpus heterophyllus Lam.* with Standard Drug Cystone

Discussion

The inhibition of aggregation assay gave a clear concentration dependent response of the test sample, with inhibition that rose from 7.30% at 100 µg/ml to 64.96% at 1000 µg/ml. However, positive control drug Cystone showed a dose-independent inhibitory effect against HCN-92 cell line with the percentage of inhibition varying from 13.87% (100 µg/ml) to 88.32% (1000 µg/ml). It is to be mentioned here that control sample was able to exceeded 50% inhibition at EBEC =800 µg/ml, whereas Cystone demonstrated > 50 inhibitions even at 600 µg/ml indicating its high potential of inhibition. The observed dose–response relationship in the test sample suggests there are bioactive principles that are responsible for its anti-aggregation activity, which typically is characteristic of phytochemical-based formulations with slow yet substantial biological activity going on. The relatively higher potency of the reference drug, however, indicates that the concentration of active principles may be lower in (Plum.) in comparison to Cystone or alternatively their mode(s) of action may differ in modulating aggregation phenomenon. Analogous comparative variations have been also reported in antimalarial studies when herbal drugs were compared with established pharmacological agents as standards, since the test extracts exhibited a promising activity and necessitated further standardization / optimization. These results therefore demonstrate that the test sample possesses therapeutic relevance, which is however in need of further phytochemical characterization, mechanistic-based studies and perhaps synergistic boosting into alignment with its efficacy relative to standard formulations such as Cystone. Taken together, our results strongly indicate that this test sample has the potential to be a potent natural anti-aggregation agent, but further validation is required before it could cross into clinical or pharmacological application.

Microscopic image of the calcium oxalate crystals by aggregation assay

Result: Microscopic images of the different concentration and standard drug results also given in figure 11

Figure 11: Microscopic images of the control, different concentration of methanolic extract of *Artocarpus heterophyllus Lam.,* and standard drug

4. Discussion

The microscopic images are also tested to confirm the results of the aggregation approach have dues to others. Microscopic images of calcium oxalate crystals, also support the % inhibition data. The outcome of the microscopic image revealed that the crystal inhibition is carried in varying proportions of methanolic extract of *Artocarpus heterophyllus Lam.* extract [100 mg/ml, 200mg / ml, 600mg / ml, 800mg / ml, and 1000mg / ml], control and standard drug (Cystone 1000mg / ml). From the control, different concentration of sample and standard drug, it showed that the size and quantity of the calcium oxalate crystals decrease when the methanolic extract of *Artocarpus heterophyllus Lam.* concentration increases. The more the visibilities of crystals are less in lower concentration and lesser visibilities of crystals is seen in higher concentration and standard drug Cystone. The results were further supported by microscopic studies and Percentage inhibition calculation, which reveals that the methanolic extract of leaves of *Artocarpus heterophyllus* Lam. Possesses anti-urolithiatic activity.

5. Conclusion

In the present study, an attempt has been made to evaluate the methanolic extract of *Artocarpus heterophyllus* Lam leaves in providing relief in kidney stone formation. In phytochemicals screening of preliminary compounds, alkaloids, flavonoids, triterpenoids, saponins tannins and phenolic compounds were identified- this botanical having anti- urolithiatic effect would be due to inhibition of important pathological events in urolithiasis. FTIR analysis comprehensively emphasized the functional group constants of these potent chemotherapeutic agents, having antioxidant, anti-inflammatory, diuretic, and antimicrobial activities, essential for treatment of urinary stone formation.

The extract showed promising in vitro anti-urolithiatic activity when tested in various experimental models, as they resulted in 60% dissolutions of the calcium oxalate crystals through titrimetric test that is proximate to the standard drug Cystone of 80%. It showed dose-dependent inhibition of calcium oxalate crystallization, with efficacy corresponding to 67.53% and 64.96 % decrease in nucleation and aggregation respectively at higher concentrations. These results were further confirmed by microscopic analysis showing that the size and quantity of crystals decreased significantly as a function of extract concentration.

In conclusion, these promising findings validate the use of *Artocarpus heterophyllus as* a potential natural anti-urolithiatic agent and support its traditional claim for the management and prevention of urolithiasis. However, validation in animal models and clinical trials is still necessary for in vivo study to investigate the mechanisms of action, develop extraction protocols, purify bioactive compounds and safety profile. Such focussed additional research will take the journey towards safe and effective plant drugs for urolithiasis, providing an auspicious decision factor in renal care.

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