

# **Invitro Evaluation of Ethanolic extract of *Acalypha Indica* Linn. Leaves for Antilithiatic Activity.**

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## ***Abstract***

*To give scientific background to the above traditional claim, the aerial parts of Acalypha Indica Linn. were evaluated for its antilithiatic activity. In the present study, aerial parts of Acalypha Indica Linn. were subjected to morphological, microscopically, phytochemical investigations and evaluated for in vivo antilithiatic activity. The plant material after defatting with pet. Ether (60-80) extracted with ethanol and then fractionated with chloroform, ethyl acetate and n-butanol. Qualitative chemical examination of various extracts revealed the presence of Alkaloids, Steroids, Flavonoids, Glycosides, Tannins, and Carbohydrates. Acute toxicity studies were carried out as per OECD guidelines and the doses were fixed at 200 mg/kg b.w. and 400 mg/kg b.w. The Ethanolic extract was evaluated for in vivo antilithiatic activity by using CCl<sub>4</sub> induced antilithiatic model. The ethanolic extract showed better effect at the dose of 10 mg/kg b.w in lowering elevated level of kidney stones in the kidney.*

**Keywords:** - *Acalypha indica Linn, Phytochemical, antilithiatic, Ethanolic extract.*

## INTRODUCTION TO HERBS:-

*Acalypha indica* (Tamil: **Kuppaimeni**) is a species of plant. It occurs throughout tropical Africa and South Africa, in India and Sri Lanka, as well as in Yemen and Pakistan. In West and East Africa the plant is used medicinally. This plant is held in high esteem in traditional Tamil Siddha medicine as it is believed to rejuvenate the body.



**Figure No. 1 Introduction to *Acalypha Indica*.**

<b>Kingdom:</b>	Plantae
<b>Division:</b>	Magnoliophyta
<b>Class:</b>	Magnoliopsida
<b>Order:</b>	Malpighiales
<b>Family:</b>	Euphorbiaceae
<b>Genus:</b>	<i>Acalypha</i>

It is named in Sanskrit –Arittamanjarie, English. –Indian Acalypha, Hindi – Kuppu; Khokali.

Habitat - Common annual shrub in Indian gardens, backyards of houses and waste place throughout the plains of India.

Parts Used - Leaves, root, stalks (young shoots) and flowers. Constituents — Alkaloids “acalypus” and “acalyphine.” Action – Cathartic, Anthelmintic, expectorant, emetic, anodyne and hypnotic.

### 2.1 Epidemiology Of Kidney stone:-

**Kidney stones (Ureterolithiasis)** result from **stones** or **renal calculi** (from Latin *ren*, *renes*, "kidney" and *calculi*, "pebbles"<sup>[1]</sup>) in the ureter. The stones are solid concretions or calculi (crystal aggregations) formed in the kidneys from dissolved urinary minerals. **Nephrolithiasis** (from Greek νεφρός (*nephros*, "kidney") and λίθος (*lithos*, "stone")) refers to the condition of having kidney stones. Urolithiasis refers to the condition of having calculi in the urinary tract (which also includes the kidneys), which may form or pass into the urinary bladder. Ureterolithiasis is the condition of having a calculus in the ureter, the tube connecting the kidneys and the bladder.

If stones grow to sufficient size before passage on the order of at least 2-3 millimeters they can cause obstruction of the ureter. The resulting obstruction causes dilation or stretching of the upper ureter and renal pelvis (the part of the kidney where the urine collects before entering the ureter) as well as muscle spasm of the ureter, trying to move the stone. This leads to pain, most commonly felt in the flank, lower abdomen and groin (a condition called renal colic). Renal colic can be associated with nausea and vomiting. There can be blood in the

urine, visible with the naked eye or under the microscope (macroscopic or microscopic hematuria) due to damage to the lining of the urinary tract.

## **2.2 Etiology of Kidney stones:-**

A stone is created when the urine does not have the correct balance of fluid and a combination of minerals and acids. When the urine contains more crystal-forming substances than the fluid can dilute, crystals can form. Normally the urine contains components that prevent these crystals from attaching to each other. However, when these substances fall below their normal proportions, stones can form out of an accumulation of crystals.

**Calcium oxalate stones:** -The most common type of kidney stone is composed of calcium oxalate crystals, occurring in about 80% of cases,<sup>[8]</sup> and the factors that promote the precipitation of crystals in the urine are associated with the development of these stones.

**Uric acid (urate):**-About 5–10% of all stones are formed from uric acid. Uric acid stones form in association with conditions that cause hyperuricosuria with or without high blood serum uric acid levels (hyperuricemia); and with acid/base metabolism disorders where the urine is excessively acidic (low pH) resulting in uric acid precipitation. A diagnosis of uric acid nephrolithiasis is supported if there is radiolucent stone, persistent undue urine acidity, and uric acid crystals in fresh urine samples

**Other types:**-Other types of kidney stones are composed of struvite (magnesium, ammonium and phosphate); calcium phosphate; and cystine. Struvite stones are also known as infection stones, urease or triple-phosphate stones. The formation of struvite stones is associated with the presence of urea-splitting bacteria, most commonly *Proteus mirabilis*.

**Treatment:Surgical Treatment-**Treatment for these stones is greatly improved, and many options do not require major open surgery and can be performed in an outpatient setting.

## **Extracorporeal Shock Wave Lithotripsy:-**

Extracorporeal shock wave lithotripsy (ESWL) is the most frequently used procedure for the treatment of kidney stones. In ESWL, shock waves that are created outside the body travel through the skin and body tissues until they hit the denser stones. The stones break down into small particles and are easily passed through the urinary tract in the urine. For most types of ESWL procedures, anesthesia is needed. Recovery time is relatively short, and most people can resume normal activities in a few days. Complications may occur with ESWL. Some patients have blood in their urine for a few days after treatment. Bruising and minor discomfort in the back or abdomen from the shock waves can occur.

## **Percutaneous Nephrolithotomy:-**

Sometimes a procedure called percutaneous nephrolithotomy is recommended to remove a stone. This treatment is often used when the stone is quite large or in a location that does not

allow effective use of ESWL. In this procedure, the surgeon makes a tiny incision in the back and creates a tunnel directly into the kidney. Using an instrument called a nephroscope, the surgeon locates and removes the stone. One advantage of percutaneous nephrolithotomy is that the surgeon can remove some of the stone fragments directly instead of relying solely on their natural passage from the kidney.

### **Ureteroscopic Stone Removal:-**

Although some stones in the ureters can be treated with ESWL, ureteroscopy may be needed for mid- and lower-ureter stones. No incision is made in this procedure. Instead, the surgeon passes a small fiberoptic instrument called a ureteroscope through the urethra and bladder into the ureter. The surgeon then locates the stone and either removes it with a cage-like device or shatters it with a special instrument that produces a form of shock wave.

### **Prevention:**

Drinking enough water to make 2 to 2.5 liters of urine per day.

A diet low in protein, nitrogen and sodium intake.

People with urinary tract infections, kidney disorders such as cystic kidney diseases, and certain rare, inherited metabolic disorders are also more likely to develop kidney stones.

Restriction of oxalate-rich foods, such as chocolate, nuts, soybeans,<sup>[28]</sup> rhubarb and spinach,<sup>[29]</sup> plus maintenance of an adequate intake of dietary calcium.

Taking drugs such as thiazides, potassium citrate, magnesium citrate and allopurinol, depending on the cause of stone formation.

Some fruit juices, such as orange, blackcurrant, and cranberry, may be useful for lowering the risk factors for specific types of stones. Orange juice may help prevent calcium oxalate stone formation, black currant may help prevent uric acid stones, and cranberry may help with UTI-caused stones.

Limit intake of caffeinated beverages, such as coffee. Avoidance of cola beverages. Avoiding large doses of vitamin C.

### **2.3 Management:**

Conservative: - Analgesia Management of pain often requires intravenous administration of NSAIDs or opioids in an emergency room setting. Orally-administered medications are often effective for less severe discomfort (NSAIDs or opioids). Intravenous acetaminophen also appears to be effective.

## **Alpha adrenergic blockers**

Alpha adrenergic blockers such as tamsulosin (Flomax) may increase the spontaneous passage of the stone by 30%. Recent studies have, however, questioned this claim, finding no benefit from these medications.

## **Urologic interventions**

Surgery is necessary when the pain is persistent and severe, in renal failure and when there is a kidney infection. It may also be advisable if the stone fails to pass or move after 30 days. Finding a significant stone before it passes into the ureter allows physicians to fragment it surgically before it causes any severe problems. In most of these cases, non-invasive extracorporeal shock wave lithotripsy (ESWL) will be used. Otherwise some form of invasive procedure is required; with approaches including ureteroscopic fragmentation (or simple basket extraction if feasible) using laser, ultrasonic or mechanical (pneumatic, shock-wave) forms of energy to fragment the larger stones.

## **Ureteral (double-J) stents**

One modern medical technique uses a ureteral stent (a small tube between the bladder and the inside of the kidney) to provide immediate relief of a blocked kidney. This is especially useful in saving a failing kidney due to swelling and infection from the stone. Ureteral stents vary in length and width but most have the same shape usually called a "double-J" or "double pigtail", because of the curl at both ends. They are designed to allow urine to drain around any stone or obstruction.<sup>[50]</sup>

## **2.6 Diagnosis & Testing method:-X-rays**

The relatively dense calcium renders these stones radio-opaque and they can be detected by a traditional X-ray of the abdomen that includes the kidneys, ureters and bladder—KUB. This may be followed by an IVP (intravenous pyelogram—intravenous urogram (IVU) is the same test by another name) which requires about 50 ml of a special dye to be injected into the bloodstream that is excreted by the kidneys and by its density helps outline any stone on a repeated X-ray. These can also be detected by a retrograde pyelogram where similar "dye" is injected directly into the ureteral opening in the bladder by a surgeon, usually an urologist. About 10% of stones do not have enough calcium to be seen on standard X-rays (radiolucent stones).

## **Computed tomography-**

All stones are detectable by CT except very rare stones composed of certain drug residues in the urine. If positive for stones, a single standard X-ray of the abdomen (KUB) is recommended. This gives a clearer idea of the exact size and shape of the stone as well as its surgical orientation.

**Other investigations typically carried out include:**

Microscopic study of urine, which may show proteins, red blood cells, bacteria, cellular casts and crystals. Culture of a urine sample to exclude urine infection

**Blood tests:** Full blood count for the presence of a raised white cell count (Neutrophilia) suggestive of infection, a check of renal function and to look for abnormally high blood calcium blood levels (hypercalcaemia). 24 hour urine collection to measure total daily urinary volume, magnesium, sodium, uric acid, calcium, citrate, oxalate and phosphate. Catching of passed stones at home (usually by urinating through a tea strainer or stonesscreen) for later examination and evaluation by a doctor.<sup>[25][26]</sup>

**Evaluation Parameters:**

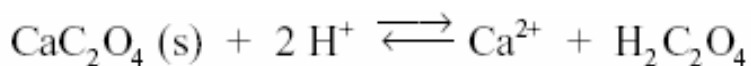
Estimation of Calcium Oxalate by Titrimetry:-

Kidney stones usually differ in chemical compositions in individual patients from varying regions from Europe, America to south East Asia and India. But Calcium oxalate is the major chemical, insoluble at the pH 7.4 (The Blood) caused due to Hypercalciuria, Hypocitraturia, Hyperoxaluria or Hyperuricosuria; thus gets deposited in the kidney, in the form of stones.

Principle:-

Weighed quantity of calcium oxalate is reacted with the extract/compound/ standard, calcium oxalate may get dissolved based on the ability of test substance to dissolve it in a semi permeable membrane, suspended in TRIS Buffer, pH 7.4. The remaining undissolved calcium oxalate is then estimated in acidic condition by titrating against 0.1 N  $\text{KMnO}_4$  to a stable light pink end point.

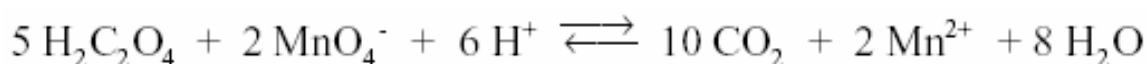
The amount of undissolved calcium oxalate is then subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually the test substance could dissolve.



Calcium oxalate

acid

oxalic acid



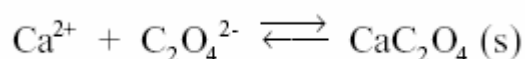
Liberated acid    standard  $\text{KMnO}_4$     acid

end point pink

## Requirements

### i) Preparation of Calcium oxalate by homogenous precipitation

Equimolar solution of Calcium chloride dihydrate (A.R) dissolved in distilled water and Sodium oxalate (A.R) dissolved in 10 ml of 2N H<sub>2</sub>SO<sub>4</sub> and distilled water, sufficient quantity is allowed to react in a beaker. Their reaction occurred was as followed-



Calcium ion    oxalate ion            calcium oxalate precipitates

The resulting precipitate was calcium oxalate which was freed from traces of sulfuric acid by ammonia solution. Washed with distilled water and dried at a temperature 60<sup>0</sup> C for 4 hours to obtain precipitate.

### ii) Preparation of the Semi permeable membrane from farm eggs:-

The semi permeable membrane of eggs lies in between the outer calcified hard shell and the inner contents rich in protein like albumin and yolk. Outer shell was removed chemically by placing the eggs in 2 M HCl for overnight, which caused complete decalcification i.e. removal of shell. Further, washed with distilled water and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while and then rinsed it with distilled water and stored in refrigerator at a pH of 7- 7.4 until further use.

## Method:

Weighed exactly 1 mg of the calcium oxalate and 10 mg of the extract/compound/standard and packed it together in semi permeable membrane by suturing as shown in Model design. This was allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer. One group served as negative control (containing only 1 mg of calcium oxalate). Placed the conical flask of all groups in an incubator, pre heated to 37<sup>0</sup> C for 2 hours, for about 7 to 8 hours. Removed the contents of semi permeable membrane from each group into a test tube. Add 2 ml of 1 N sulfuric acid and titrated with 0.9494 N KMnO<sub>4</sub> till a light pink color end point obtained.

1ml of 0.9494 N KmnO<sub>4</sub> equivalent to 0.1898 mg of Calcium. Percentage dissolution of calcium oxalate by various groups is shown in figure.

### 2. Estimation of Calcium Phosphate by Colorimetry:-

Calcium phosphate is the second major chemical present in the kidney stones, insoluble at the pH 7.4 (The Blood), caused due to Renal tubular acidosis or Uric acid; thus gets deposited in the kidney, in the form of stones

### Principle:

Weighed quantity of calcium phosphate is reacted with the extract/ compound/ standard, calcium phosphate may get dissolved based on the ability of test substance to dissolve it in a semi permeable membrane, suspended in TRIS Buffer, pH 7.4. The remaining undissolved calcium phosphate is then reacted with Molybdic-Sulfuric Reagent and Reducing solution to form a stable greenish-blue colored complex, whose optical density is measured at 600-750 nm colorimetrically This method is a modification of Fiske and Subbarow method and Kuther and Cohen method by Gomori et.al.

A Standard calibration curve drawn by plotting the O.D.values of various groups against concentration. The amount of undissolved calcium phosphate is determined by extrapolation, which is then subtracted from the total quantity of calcium phosphate used for the experiment in the beginning, to know how much quantity of calcium phosphate actually the test substance could dissolve.

Requirements:-

i) Preparation of Calcium phosphate by homogenous precipitation:-

Equimolar solution of Calcium chloride dihydrate (A.R) dissolved in distilled water and Disodium hydrogen phosphate (A.R) dissolved in 10 ml of (2N H<sub>2</sub>SO<sub>4</sub>) and distilled water.



Calcium ion      phosphate ion      calcium phosphate precipitate

The resulting precipitate was calcium phosphate which was freed from traces of sulfuric acid by ammonia solution .Washed with distilled water and dried at a temperature 60<sup>0</sup> C for 4 hours.

ii) Preparation of the Semi permeable membrane from farm eggs

This is prepared in the same way as described earlier.

iii) Preparation of Molybdate-sulphuric acid reagent

This is prepared by mixing 2 parts of 5% w/w solution of Sodium molybdate (A.R), 1 part of 10 N sulfuric acid and 1 part of distill water.



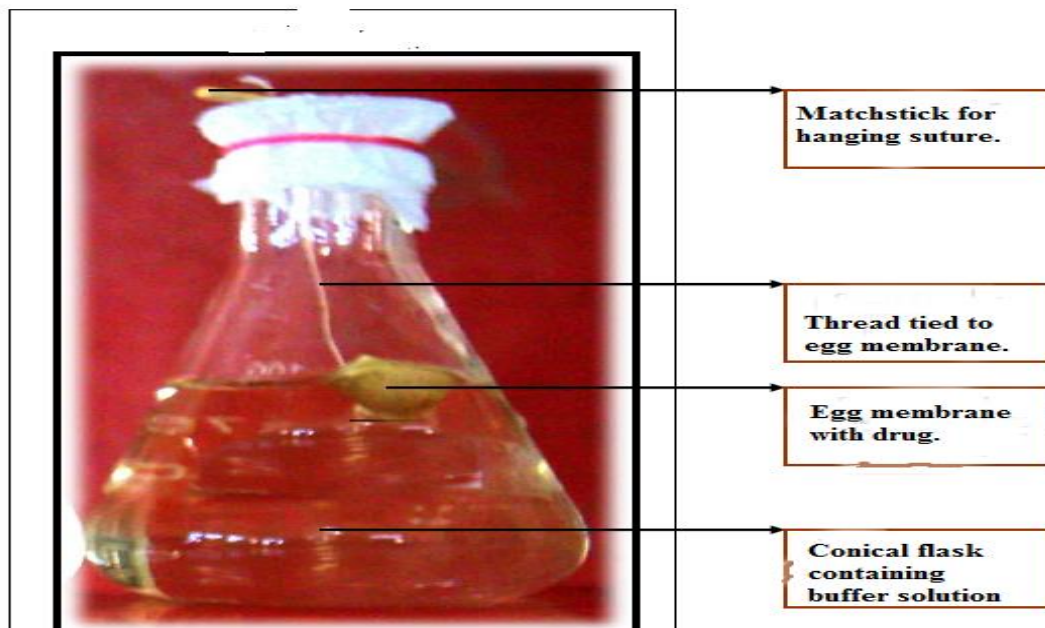
#### iv) Preparation of reducing solution

1 g. of *p*-Phenylene diamine is dissolved in 100 ml of 3 % w/w solution of Sodium bisulfite to get the required solution.

**Method:** - Weighed exactly 1 mg of the calcium phosphate and 10 mg of the extract/compound/standard and packed it together in semi permeable membrane by suturing. This was allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer. One group served as negative control (contained only 1 mg of calcium phosphate) Placed the conical flask of all groups in an incubator, pre heated to 37<sup>0</sup> C for 2 hours, for about 7-8 hours Removed the contents of semi permeable membrane from each group into a test tube Added 2 ml of 1 N sulfuric acid, 2.5 ml of Molybdic-sulphuric acid reagent, 1 ml of Reducing solution and made up the volume to 10 ml using distill water. Standard dilutions of calcium phosphate were prepared, (200, 400, 600, 800 and 1000 µg/ml) containing 2.5 ml of Molybdic-sulphuric acid reagent, 1 ml of Reducing solution and made up the volume to 10 ml using distilled water respectively. Measured the optical density of standard dilutions and for the groups under study in colorimeter using the Filter no.67. The undissolved calcium phosphate was determined from the standard calibration curve by extrapolation.

#### **Result and discussion:**

Our study revealed that the Ethanolic extract of the *Acalypha indica* Linn. Leaves contain Alkaloids, Steroids, Flavonoids, Glycosides, Tannins, and Carbohydrates. The Ethyl acetate fraction of the leaves showed significant antilithiatic activity in terms of reduction in weight of calcium in titrimetric evaluation may be due to presence of Flavonoid in the ethyl acetate extract. While Cystone which is prescribed widely for the treatment of urinary and renal calculi showed an excellent antilithiatic activity in terms of reduction in weight of calcium in titrimetric evaluation, (Table-1), (Histogram-1). While on another hand chloroform fraction has showed least action. Our study needs further research work for the further isolation of the principle active constituent from the ethyl acetate fraction which is mainly responsible for the antilithiatic activity.

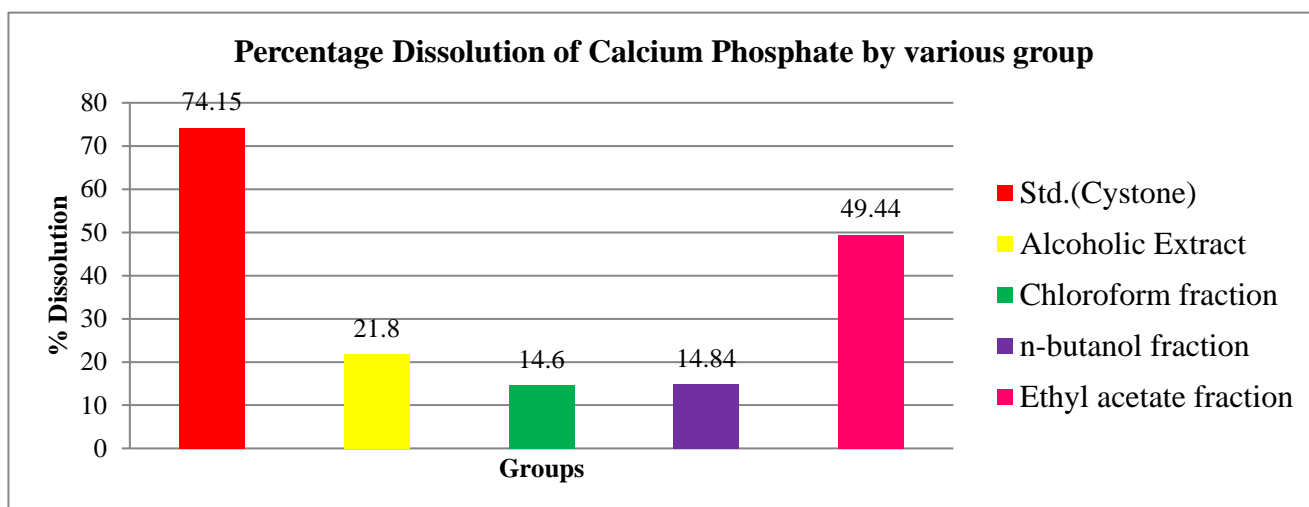


**Figure No. 7: Conical flask containing buffer & egg membrane suspended in it.**

Table No:1

Group	Vol. of St.KMno4	Wt. of cal.Estimated	Wt. of Cal. reduced	% Dissolution
Control	4.3	0.9845	-----	-----
Std.(Cystone)	2.3	0.5321	0.3946	74.15
Alcoholic Extract	3.4	0.7315	0.1596	21.80
Chloroform fraction	4.1	0.7601	0.1110	14.60
n-butanol fraction	3.9	0.7519	0.1128	14.84
Ethyl acetate fraction	2.8	0.5996	0.2965	49.44

Histogram-1



**Figure No.8 : Estimation of undissolved  $\text{CaPO}_4$  by titration method.**

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