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# *IN-VITRO* ANTIUROLITHIATIC ACTIVITY OF MACERATED AQUEOUS EXTRACT OF *TERMINALIA CHEBULA* BY USING TITRIMETRY METHOD

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**Keywords:**

Titrimetry method, *Terminalia chebul*a, Cystone, Calcium oxalate, Kidney stone

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**ABSTRACT:** Urolithiasis is a complex process that occurs from the various physiochemical event, including super-saturation aggregation, nucleation, growth, nucleation within the kidney. Data from different types of studies revealed that phytomedicines useful as an alternative remedy for the management of Urolithiasis. *Terminalia chebula* has been reported to possess antiurolithiatic property. The fruits were extracted in aqueous by maceration. *In-vitro* study was conducted to study the anti-urolithiatic effect of fruits of a macerated aqueous extract with standard drug Cystone. The titrimetric method was performed to evaluate the dissolution of stone crystal. Aqueous extract of fruits of *Terminalia chebula* proved a significant effect compared with standard drug Cystone. Aqueous extract of fruits of *Terminalia chebula* dissolves 48% crystal while Cystone dissolved 62% calcium oxalate crystal in an *in-vitro* study.

**INTRODUCTION:** Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. Medicinal plants have got a significant role in saving the lives of rural area people. Urolithiasis is a multi-factorial process that may relate to diet, urinary tract infection, altered urinary solutes, and colloids decreased urinary drainage and urinary stasis, prolonged immobilization, Randall's plaque, and microliths, *etc.* [1] Bacterial infection may induce stone formation by crystal adherence [2]. Most of the urea-splitting organisms belong to species Proteus but, organisms such as Pseudomonas, Staphylococcus, *Escherichia coli*, and even Mycoplasma were reported to be capable of producing urease [3, 4].

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Oxalic acid combines with calcium to form calcium oxalate crystals, which deposit in the kidneys. In Ayurvedha, Unani and Sidha medicine many herbs are used in the management of urolithiasis. *Terminalia chebula* (family: Combretaceae) locally known as 'Myrobalan'. It is found in the sub-Himalayan tracks from Ravi to West Bengal, Assam.

It is a tree, 15-25 m height and 1.5-2.5 m diameter, rounded crown with spreading branches and ovate leaves. It has a yellow-white flower in the terminal spike; the fruits are hard and stony with a single seed, which is light yellow.

Fruits are mainly used as astringent, and also used as anthelmintic, nervine, expectorant, tonic, carminative, appetite stimulant. Fruits pulp is used to cure bleeding, used for the treatment of piles and external ulcers. It is an ingredient of Ayurvedic preparation Triphala used for the treatment of a variety of ailments [5].

## MATERIALS AND METHODS:

**Plant Collection and Identification:** Fruits are collected from the local Ayurvedic shop at Cherthala in Alappuzha district. The fruits were identified and authenticated by Dr. Akhila, Associate Professor and Head of Pharmacognosy department, KVM College of Pharmacy, Cherthala.

**Plant Extraction:** *Terminalia chebula* fruits were collected, washed, and dried. Size reduced by the mechanical grinder and passed through sieve no. 20. The drug material is taken in a stoppered container with about 750 ml of chloroform water and allowed to stand seven days in a warm place with frequent shaking. The mixture of crude drug-containing solvent was filtered until most of the liquid drains off. The filtrate and the washing are combined to produce 1000 ml of the solution and evaporated by vacuum evaporator [6].

**Preliminary Phytochemical Evaluation:** Preliminary phytochemical analysis was carried out on the macerated ethanolic and water extracts using the standard procedure to identify the constituents present by characteristics color changes.

**Evaluation of Antiurolithiatic Activity by Calcium Oxalate Dissolution Method:**

**Titrimetric Method:** The titrimetric method is one of the dissolution methods; the artificial preparation of calcium oxalate crystal was taken in the egg semi-permeable membranes act as a control and added different plant extracts and standard Cystone. Then it was immersed in the 0.1 M Tris buffer and incubated at 37 ºC for 2 h. After 2 h removal of content of semi-permeable membrane and add 2 ml of 1 N Sulphuric acid titrated against 0.9494 N potassium permanganate till a light pink color endpoint is obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment, in the beginning, to know the total quantity of dissolved calcium oxalate by various extracts [7-10]. The dissolution method involved three steps

- Preparation of experimental kidney stones (calcium oxalate stones) by homogenous precipitation.
- Preparation of semi-permeable membrane from eggs.
- Estimation of calcium oxalate by titrimetric method.

**Step 1: Preparation of Experimental Kidney Stones (Calcium Oxalate Stones) by Homogenous Precipitation:** 1.34 g of sodium oxalate was dissolved in 100 ml of 2 N sulphuric acid and 1.47 g of calcium chloride dihydrate was dissolved in 100 ml distilled water. Both were mixed equally in a beaker to precipitate calcium oxalate. The precipitate free from traces of sulphuric acid by ammonia solution. Washed the precipitates with distilled water and dried at 60 ºC for 4 h.

**Step 2: Preparation of Semi-permeable Membrane from Eggs:** The semi-permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin and yolk. The apex of eggs was punctured by a glass rod to squeeze out the entire content. Empty eggs were washed thoroughly with distilled water and placed in a beaker consisting of 2 M hydrochloric acid for an overnight, which causes complete decalcification. Further, it was washed with distilled water, placed it in ammonia solution for neutralization of acid traces in the moistened condition for a while and rinsed it with distilled water and stored in the refrigerator at pH of 7-7.4

**Step 3: Estimation of Calcium Oxalate by Titrimetry:** The dissolution percentage of calcium oxalate was calculated by taking exactly 1 mg of calcium oxalate and 10 milligrams of plant extract, 10 mg of Cystone (standard) packed it together with an egg semi-permeable membrane. This was allowed to suspend in a conical flask containing 100 ml 0.1 M Tris buffer. Egg semi-permeable membrane 1 mg of calcium oxalate acts as a control. The entire conical flask with a semi-permeable membrane was kept in an incubator at 37 ºC for 2 h.

Remove the contents of semi-permeable into a separate test tube, add 2 ml of 1N sulphuric acid to each test tube and titrate with 0.9494 N potassium permanganate till a light pink color endpoint was obtained. The amount of remaining calcium oxalate is subtracted from the total quantity used in the experiment, in the beginning, to know the total quantity of dissolved calcium oxalate by various extracts.

Each ml of 0.9494 N potassium permanganate equivalents to 0.1898 mg of calcium oxalate. The percentage of dissolution was calculated as

Dissolved calcium oxalate = (undissolved calcium oxalate-total quantity used in the experiment in the beginning)

Percentage dissolution = Dissolved calcium oxalate × 100

**RESULTS:** Preliminary phytochemical analysis of macerated aqueous dried fruits extract of *Terminalia chebula* revealed the presence of some secondary metabolites in **Table 1**.

**TABLE 1: PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *TERMINALIA CHEBULA* FRUITS EXTRACT**

| Phytoconstituents | Aqueous extract |
|---|---|
| Alkaloids | + |
| Flavanoids | + |
| Glycosides | + |
| Steroid | + |
| Tannins | + |
| Phenols | + |
| Terpenoids | + |
| Saponin | + |
| Resins | - |
| Carbohydrate | + |

***In-vitro* Urolithiatic Study:** The percentage of dissolution of aqueous extract was calculated and from dissolved and undissolved calcium oxalate.

**TABLE 2: *IN-VITRO* ANTIUROLITHIATIC STUDY OF *TERMINALIA CHEBULA* FRUITS EXTRACT**

| Drug | Dissolved calcium oxalate (mg) | % dissolution |
|---|---|---|
| Cystone | 0.62 ± 0.005 | 62% |
| Aqueous extract | 0.48 ± 0.005 | 48% |

**N=3 Mean ± SD**

The result of the present study indicate the aqueous extract of *Terminalia chebula* fruits shows significant calcium oxalate crystallization inhibition of Plant extract 48%, and Cystone, prescribed medicine for renal caliculi showed the highest inhibition 62%.

**DISCUSSION:** The qualitative evaluation of herbal extract contains different phytochemicals with biological activity that can be of variable therapeutic index. Different phytochemicals have been found to possess a wide range of activities that may help in protection against chronic diseases. For example, alkaloids and flavonoids anti-inflammatory activity.

The qualitative analysis of aqueous extracts of *Terminalia chebula* was carried out, and extract shows the presence of alkaloids, flavonoids, steroids, phenols, saponin, carbohydrate **Table 1**. The saponins are possessed anti-crystallization property by disaggregating the suspension of mucoproteins, promoters of crystallization. Anti-urolithiatic activity of this plant may be due to the presence of these phytocompounds.

The present *in-vitro* study recorded that the plant extract has moderate anti-urolithiatic activity with a maximum inhibition of 48% of aqueous extract, and using standard drug (Cystone) drug has potent anti-urolithiatic activity. These two results compared and confirmed the plant extract have moderate anti-urolithiatic activity than standard drug. In the titrimetric method, shows dissolved calcium oxalate in mg is low (0.48 mg) as compared with standard (0.62 mg). Compare the percentage of the dissolution of aqueous extract of *Terminalia chebula* with standard shows less percentage of dissolution than standard.

**CONCLUSION:** Fruits of *Terminalia chebula* selected for this study. Qualitative chemical tests showed alkaloids, flavonoids. The *in-vitro* anti-urolithiatic study of aqueous extract of fruits of *Terminalia chebula* through the titrimetric method has shown significant action on urinary calculi. Titrimetric estimation measures undissolved calcium oxalate by using potassium permanganate with aqueous extract of test drug showed dissolution of 48%, respectively, which was compared to the standard drug (Cystone) 62%. This drug may be beneficial in the treatment of urolithiasis.

In the future, these drugs can be performing *in-vivo* and clinical study beneficial for people with avoiding the adverse effect of modern medicinal drugs.

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**CONFLICTS OF INTEREST:** Nil

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