



**COMPARISON OF *IN VITRO* ANTIUROLITHIATIC ACTIVITY OF *MEYNA
LAXIFLORA* AND *TECTONA GRANDIS*****AHIRE SD^{*}, ZALTE AG AND GULECHA VS**

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Urolithiasis is a term used to refer calculi or stones formed in urinary tract. It includes the development of calcifications in the urinary system, generally in the kidneys or ureters, but may also affect the bladder or urethra. It is a severe, devastating problem in all societies all over the world, affecting almost, 12% of the population and men are three times more prone than women. It is more predominant between the ages of 20 and 40 in both sexes.

The present study was undertaken to evaluate the in vitro antiurolithiatic activity of the medicinal plant *Meyna laxiflora* and *Tectona grandis*. Alcoholic extracts of both the plants showed their maximum efficiencies in the dissolution of calcium oxalate crystals. Our results have clearly indicated that the alcoholic extracts of *Meyna laxiflora* and *Tectona grandis* were quite promising for further studies in this regard.

Keywords: *Meyna laxiflora*, *Tectona grandis*, Alcoholic extracts, Cystone, Calcium oxalate**INTRODUCTION**

Urolithiasis is characterized by the formation of a stone in the kidneys or urinary tracts. A large number of people, nearly 4–15% of the human populations are suffering from urinary stone problem all over the globe [1]. In addition, the recurrence rate is high, being more than 50% after 10 years. The occurrence in some

areas is so alarming that they are known as 'Stone Belts'. The area of high incidence of urinary calculi include British islands, Scandinavian countries, Central Europe, Northern Australia, Northern India, Pakistan, Mediterranean countries [2]. The crystals of calcium oxalate (CaOx) are the primary constituent of more than 60% of

the majority of human kidney stones; they exist in the form of CaOx monohydrate (COM) and CaOx dehydrate (COD) [3].

Meyna laxiflora is a small or medium size tree, commonly known as Alu, Huloo belonging to family Rubiaceae which having traditional importance for its medical uses, for treatment of Inflammation, Gastrointestinal disorder etc [4]. It is distributed in tropical and subtropical region all over the world. In India it is available in Assam, Bengal, Konkan, Deccan etc and widely available throughout Satpuda region mainly in Sawarimal, Umarpata, Morkaranja and kondaibari villages of Nandurbar district [5]. The chemicals reported from the plant belong to different classes such as carbohydrates, starch, proteins, tannins, saponins and alkaloids. The remarkable pharmacological properties include anti-diabetic, antioxidant, antibacterial, antifungal and many more activities [6].

Tectona grandis Linn (Teak), is locally known as Sagwan, belongs to Lamiaceae family. It is one of the most valuable timber in the world, due to its beautiful surface and its resistance to termite and fungal damage [7]. The main active ingredient compounds that are responsible for this action are tectoquinone, lapachol and deoxylapachol. Naphthoquinones, anthraquinones and isoprenoidquinones are

abundant metabolites in teak [8]. In addition to these, teak contains several other phytochemicals such as triterpenoids, steroids, lignans, fatty esters and phenolic compounds [9]. Pharmacologically, the plant has been investigated for antioxidant, anti-inflammatory, anti-pyretic, cytotoxic, analgesic, hypoglycemic, wound healing and antiplasmodial activities [10].

The present study was carried out for comparison antiurolithiatic activity of *Meyna laxiflora* and *Tectona grandis*.

MATERIAL AND METHODS

Plant material collection and authentication

The plants were collected from Nampur region of Satana, Dist: Nashik, Maharashtra, India, in and authenticated by Dr. D. G. Shimpi, HOD, Department of Botany, Bytco College, Nashik road, Nashik.

The collected samples were cleaned, shade dried and pulverized by using mechanical pulverizer for size reduction to make coarse powder.

The plant material was thoroughly cleaned to remove adhered dirt, followed by rinsing with distilled water, shade dried and pulverized in a mechanical grinder to obtain coarse powder. The powder was used for further studies.

Experimentally kidney stones were prepared by homogenous precipitation

method. Semi - permeable membrane was removed chemically by placing the eggs in 2M HCl for overnight, which caused complete decalcification. Then the membranes were washed thoroughly with distilled water, stored in refrigerator at a pH of 7-7.4 [11, 12].

Preparation of Standard Solution:

A poly herbal formulation such as Cystone was selected and tablets were placed in absolute ethanol for removing colour coating and were crushed into powder form. The powder was dispersed into 100ml of distilled water and filtered. Filtrate was used as positive control [13, 14].



Figure 1(a) Eggs kept for calcification



Figure 1(b): Decalcified Eggs

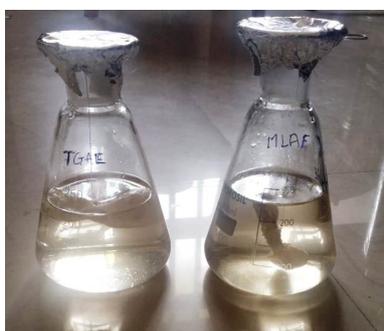


Figure 1(c): Egg membrane with contents.

Figure 1: In vitro experimental model setup to evaluate antiurolithiatic activity

RESULTS AND DISCUSSION

Estimation of CaOx and CaPo₄ by Titrimetry:

Comparison of antilithiatic activity by using standard Cystone tablets were carried out by taking control, standard, test groups (Table 1).

Calcium Oxalate:

All the models were prepared by packing in semi permeable membrane as mentioned above. The membranes were sutured and were allowed to suspend in 100ml of 0.1M Tris buffer. All the flasks were subjected to incubation, preheated to 37°C for 7hrs for 3days. After 3 days content of each membrane was collected in different test tubes. 2ml of 1N Sulphuric acid was added to each test tube and titrated with 0.9494N KMnO₄ till the colour disappears. The amount of undissolved Calcium oxalate is subtracted from the total quantity used in

the experiment in the beginning; to know much quantity of Calcium oxalate actually test substances could dissolve (Table 2).

All the models were prepared by packing in semi permeable membrane as mentioned above. The membranes were sutured and were allowed to suspend in 100ml of 0.1M Tris buffer. All the flasks were subjected to incubation, preheated to 37°C for 7hrs for 3days. 4ml of 1N H₂SO₄ and 3ml of molybdate-sulphuric acid reagent, 1ml of reducing solution were added and kept aside for 2hrs. Change in colour intensity was measured against 620nm spectrophotometrically. The amount of undissolved Calcium Phosphate is subtracted from the total quantity used in the experiment in the beginning, to know much quantity of Calcium phosphate actually test substances could dissolve (Table 3, 4).

$$\% \text{ Inhibition} = \frac{(\text{weight of precipitate in blank set}) - (\text{Weight of precipitate in experimental set})}{(\text{Weight of precipitate in blank set})} \times 100$$

Table 1: *In-vitro* Antilithiatic activity of CaOx Dissolution model

Control	Standard	<i>Meyna laxiflora</i>	<i>Tectona grandis</i>
1ml (1mg/1ml) CaOx +1ml water	1ml CaOx +1ml (400 mg/ml) Cystone	1ml CaOx +1ml (100mg/ml) MLAE	1ml CaOx +1ml (100mg/ml) TGAE
		1ml CaOx +1ml (200mg/ml) MLAE	1ml CaOx +1ml (200mg/ml) TGAE
		1ml CaOx +1ml (300mg/ml) MLAE	1ml CaOx +1ml (300mg/ml) TGAE
		1ml CaOx +1ml (400mg/ml) MLAE	1ml CaOx +1ml (400mg/ml) TGAE

MLAE: *Meyna laxiflora* alcoholicextract, TGAE: *Tectona grandis* alcoholic extract, CaOx:Calcium Oxalate

Table 2: In-vitro Antilithiatic activity of CaPo₄ Dissolution models

Control	Standard	<i>Meyna laxiflora</i>	<i>Tectona grandis</i>
1ml (1mg/1ml) CaPo ₄ +1ml water	1ml CaPo ₄ +1ml (400 mg/ml) Cystone	1ml CaPo ₄ +1ml MLAE 100mg/ml	1ml CaPo ₄ +1ml TGAE 100mg/ml
		1ml CaPo ₄ +1ml MLAE 200mg/ml	1ml CaPo ₄ +1ml TGAE 200mg/ml
		1ml CaPo ₄ +1ml MLAE 300mg/ml	1ml CaPo ₄ +1ml TGAE 300mg/ml
		1ml CaPo ₄ +1ml MLAE 400mg/ml	1ml CaPo ₄ +1ml TGAE 400mg/ml

MLAE: *Meyna laxiflora* alcoholic extract, TGAE: *Tectona grandis* alcoholic extract, CaPo₄: Calcium phosphate

Table 3: Percentage inhibition of CaOx mineralisation in dissolution models

Control	Standard	<i>Meyna laxiflora</i>	<i>Tectona grandis</i>
0.0	90.55±1.27%	83.45±1.27%	81.76±1.28%
		85.94±1.26%	83.13±1.27%
		86.72±1.29%	84.94±1.26%
		88.47±1.32%	85.47±1.29%

Table 4: Percentage inhibition of CaPo₄ mineralisation in dissolution models

Control	Standard	<i>Meyna laxiflora</i>	<i>Tectona grandis</i>
0.0	90.55±1.27%	83.57±1.27%	80.57±1.32%
		84.93±1.28%	81.74±1.32%
		86.65±1.28%	82.68±1.33%
		87.38±1.29%	83.53±1.34%

The phyto chemical constituents present in the extract can be held responsible for different medicinal activities of the plant. Cystone a prescribed medicine for renal calculi showed highest inhibition of both CaOx and CaPo₄ mineralization. MLAE showed almost similar inhibition whereas TGAE showed considerably less inhibition when compared with the standard cystone at different concentrations.

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