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In-vitro anti-lithiatic activity study of *Meyna laxiflora* seeds and *Tectona grandis* bark

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***Abstract*---**This study involves the evaluation of *Meyna laxiflora* seeds and *Tectona grandis* bark for antiurolithatic activity. Homogeneous precipitation method was used for *In-vitro* anti-lithiatic activity on generated calcium oxalate crystals. Extracts were compared with synthetic drug Spironolactone, polyherbal formulation Cystone, other extracts and furosemide. Extracts like aqueous extract, pet ether extract, chloroform extract, methanol extract and aqueous extract were used for study at different concentrations.

Keywords*---Meyna laxiflora, Tectona grandis,* Cystone, extraction.

1. Introduction

Urolithiasis is a disease known from ancient times, but still now the causes responsible for the formation of some of the different kinds of stones are unknown, and consequently efficient therapeutic treatments have not yet been developed (Ghaisas M.M., 2008). There are several types of stones, most commonly consisting of calcium phosphates and oxalates; others are composed of magnesium, ammonium phosphate (struvite), uric acid or cystine. Struvite stones are associated with urinary tract infections, often of urease-secreting bacteria that increase urinary ammonium concentration (Asif M., 2011). Calcium stones may form for a number of reasons, including hypercalciuria and hyperoxaluria. Hypercalciuria may arise from changes to calcium resorption from bone, or renal and gastrointestinal tract handling of calcium; these are often associated with changes to parathyroid hormone secretion. Hyperoxaluria can arise from increased production or increased gut absorption (Qazi Majaz, A., 2015). Other factors causing hypercalciuria include renal tubular acidosis or conditions that reduce the urinary concentrations of certain inhibitors, such as citrate and magnesium. Uric acid and cystine stones can form as a result of increased production or urinary concentration of the primary constituents (Siddiqui, W. A., 2018). The extracts of *Meyna laxiflora and Tectona grandis bark* have inhibitory effect on CaOx crystallization thus may be beneficial in the treatment of urolithiasis. But there is a need of detailed investigation in elaborated preclinical experimentation to establish the use of plant as antiurolithiatic agent.

2. Experimental Work-

Model-1

The experiment consisted of the following test tubes of 10 ml capacity and marked the tubes as control and tests into 8 groups, each group has 6 test tubes, in each tube 1ml of calcium chloride anhydrous and 1ml sodium oxalate were added to the tubes and 2 ml of Tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) adjusted at 7.4 pH which to the kidney pH and incubated at 36.7^{0}C over night. The next day the test tubes were centrifuged for 10min to decant to remove top liquid layer. The calcium oxalate crystal formed in the test tube were checked using the compound microscope under 45x magnification, the crystal formed were resembling the prisms shape, to this 5ml (5mg/ml) equivalent to 25mg to each test tube of the different sequential extracts *Meyna laxiflora* seeds and *Tectona grandis* bark and were induced to the tubes and at the same quantity the synthetic drugs Spironolactone, Furosemide and the Poly herbal formulation Cystone were administered to the test tube, all the above treating agents was administered as aqueous suspension using Tween 60 as suspending agent and again it was incubated 36.7^{0}C for 3 days on the fourth day all the test tubes were taken and checked for dissolution of the crystals under the microscope (Mishra, H., 2022).

Groupings:
Group- I - generated calcium oxalate crystals and referred as control,
Group- II - generated calcium oxalate crystals + 5ml Furosemide,

Group- III - generated calcium oxalate crystals + 5ml Spironolactone,
Group- IV - generated calcium oxalate crystals + 5ml Cystone,
Group- V - generated calcium oxalate crystals + 5ml Pet Ether Extract of *Meyna laxiflora* seeds,
Group- VI- generated calcium oxalate crystals + 5ml Pet Ether Extract of *Tectona grandis* bark
Group-VII - generated crystals + 5ml Chloroform Extract of *Meyna laxiflora* seeds,
Group-VIII- generated crystals + 5ml Chloroform Extract of *Tectona grandis* bark,
Group-IX - generated crystals + 5ml Alcohol Extract of *Meyna laxiflora* seeds,
Group-X- generated crystals + 5ml Alcohol Extract of *Tectona grandis* bark
Group-XI - generated crystals + 5ml Aqueous Extract of seeds of *Meyna laxiflora.*
Group-XII- generated crystals + 5ml Aqueous Extract of and *Tectona grandis* bark.

The *In-vitro* lithiatic activity which has been carried, where the calcium oxalate crystals were generated by the sodium oxalate and calcium chloride on incubation at 36.7^0C with tris phosphate buffer at 7.4 pH. The generated crystals were treated with the different agents mentioned above in experimental part into twelve groups and group-I kept as control and others groups as treated, the estimation of calcium and oxalate were carried out, on comparison, the group-I with other groups found that the alcoholic extract of plants *Meyna laxiflora* and *Tectona grandis,* has shown significant and better action in dissolving the crystals, the cystone and spironolactone which has shown restrained activity, where as other extract and furosemide were much equivalent to the control. On detail *In-vitro* study, it was found that the alcoholic extract of the plants *Meyna laxiflora* and *Tectona grandis* has shown significant anti-lithiaticactivity in dissolution of regenerated calcium oxalate crystals.

Model-2

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 (Rai, K., 2022).

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.

Figure 1: Eggs kept for calcification

Figure 2: Decalcified Eggs

In vitro experimental model setup to evaluate antiurolithiatic activity-Estimation of CaOx and $CaPo_4$ by Titrimetry-

Comparison of Antilithiatic activity by using standard Cystone tablets were carried out by taking control, Standard, Test groups.
MLAE: *Meyna laxiflora* alcoholic extract, TGAE: *Tectona grandis* alcoholic extract, CaOx: Calcium Oxalate

Fig no 3: Egg membrane with contents

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

Control	Standard	*Meyna laxiflora*	*Tectona grandis*
1ml (1mg/1ml)	1ml CaOx +1ml	1ml CaOx +1ml (100mg/ml) MLAE	1ml CaOx +1ml (100mg/ml) TGAE

CaOx +1ml water	(400 mg/ml) Cystone	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

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 37ºC 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 $KMnO_4$ 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.

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 37ºC for 7hrs for 3days. 4ml of 1N $H2SO_4$ 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 2: *In-vitro* Antilithiatic activity of $CaPo_4$ Dissolution models

Control	Standard	*Meyna laxiflora*	*Tectona grandis*
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

3. Results

Model 1- Model 1- On detail *In-vitro* study, it was found that the alcoholic extract of the plants *Meyna laxiflora* and *Tectona grandis* has shown significant anti-lithiaticactivity in dissolution of regenerated calcium oxalate crystals.

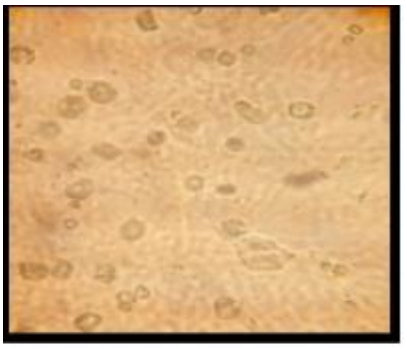

Fig: 4) Generated Calcium oxalate

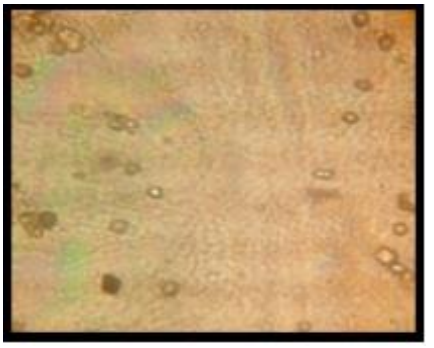

Fig: 5) Furosemide (5mg/ml) crystals as Control

Group I: (Fig: 4) The huge number of calcium oxalate crystals creation were seen
Group II: (Fig: 5) The huge numbers of calcium oxalate crystals creation were notdissolved by the drug

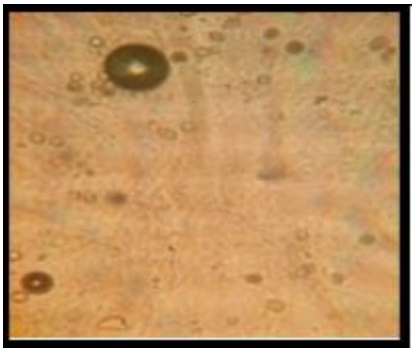

Fig: 6) Spironolactone (5mg/ml)

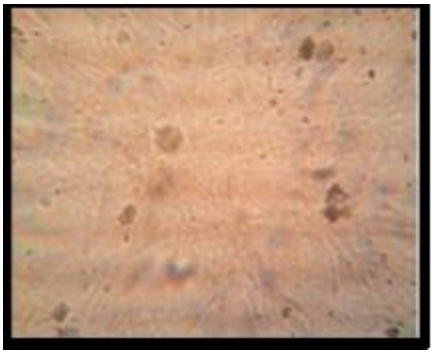

Fig: 7) Cystone (5mg/ml)

Group III: (Fig: 6) The number of calcium oxalate crystals dissolutions was less by the drug.
Group IV: (Fig: 7) The number of calcium oxalate crystals dissolution was more by the polyherbalformulation

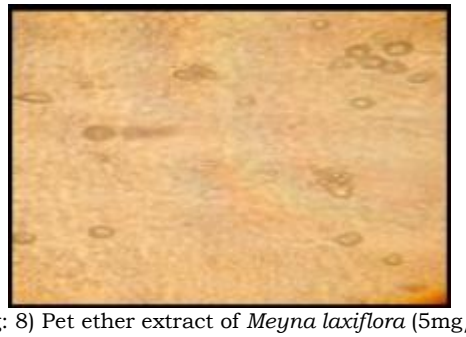

Fig: 8) Pet ether extract of *Meyna laxiflora* (5mg/ml)

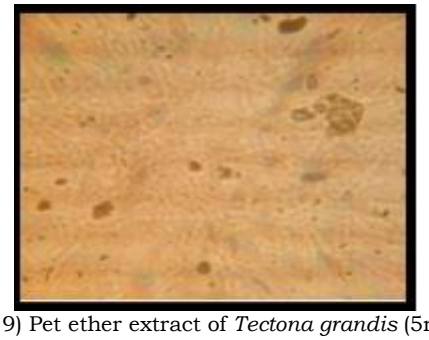

Fig: 9) Pet ether extract of *Tectona grandis* (5mg/ml)

Group V: (Fig: 8) The huge number of calcium oxalate crystals creation found undissolved by the extract.

Group VI: (Fig: 9) The number of calcium oxalate crystals creation found undissolved by the extract.

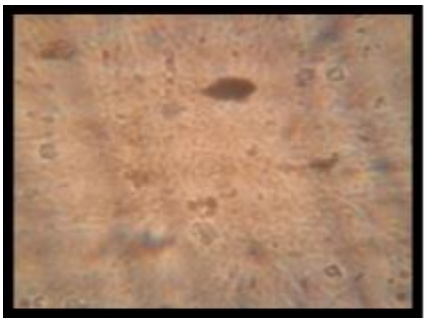

Fig: 10) Chloroform Extract of *Meyna laxiflora*

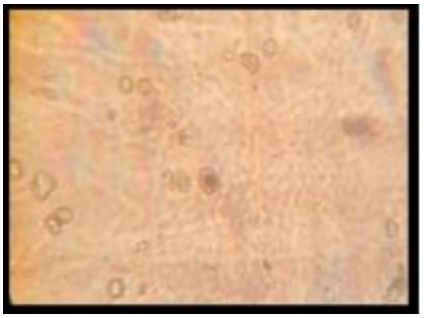

Fig: 11) Chloroform Extract of *Tectona grandis*

Group VII: (Fig: 10)The number of calcium oxalate crystals was dissolved maximum by the extract.

Group VIII:(Fig: 11) The number of calcium oxalate crystals was dissolved less by the extract.

Fig: 12) Alcohol Extract of *Meyna laxiflora* (5mg/ml)

Fig: 13) Alcohol extract of *Tectona grandis* (5mg/ml)

Group IX: (Fig: 12) The number of calcium oxalate crystals was dissolved maximum by the extract.

Group X: (Fig: 13) The number of calcium oxalate crystals was dissolved maximum by the extract

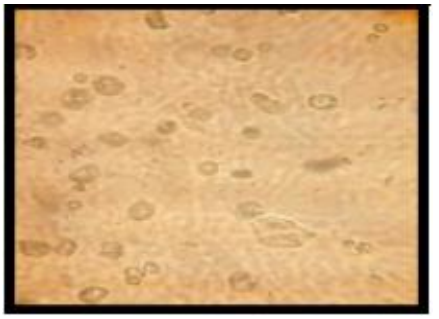

Fig: 14) Aqueous Extract of *Meyna laxiflora* (5mg/ml)

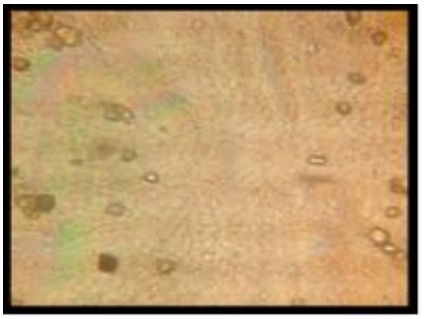

Fig: 15) Aqueous Extract of *Tectona grandis* (5mg/ml)

Group XI: (Fig: 14)The number of calcium oxalate crystals creation found undissolved by the extract

Group XII: (Fig: 15) The number of calcium oxalate crystals creation found undissolved by the extract- Model-2

Table 3: Percentage inhibition of CaOx mineralization in dissolution models

Control	Standard	*Meyna laxiflora*	*Tectona grandis*
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%

Table4: Percentage inhibition of $CaPO_4$ mineralization in dissolution models

Control	Standard	*Meyna laxiflora*	*Tectona grandis*
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%

4. Discussion

In the present study the plants *Meyna laxiflora and Tectona grandis* were selected for the antiurolithiatic activity. The successive extractions of plants were carried out and the extracts were evaluated for *in-vitro* anti-urolithiatic activity (Salem, P. P., 2020). Extraction is the first step to separate the desired natural constituents from the crude material (Wang, R., 2020). Extraction process involves solvent penetration into the crude drug, this solvent then diffuses into crude drug and all the constituents are then diffused into the extract. Similarly, extraction of *Meyna laxiflora* seeds powder and *Tectona grandis* bark were carried out using soxhlet apparatus by using four solvent systems, Methanol, water, chloroform and petroleum ether extracts (Vyas, P., 2022). The different solvent extracts of *Meyna laxiflora* seeds and *Tectona grandis* bark were executed on generated calcium oxalate crystals by homogenous precipitation method for *In-vitro* anti-lithiatic activity. Cystone a prescribed medicine for renal calculi showed highest inhibition of both CaOx and $CaPO_4$ mineralization (Bagali, R. S., 2020). MLAE showed almost similar inhibition whereas TGAE showed considerably less inhibition when compared with the standard Cystone at different concentrations. The Methanolic extracts of*Meyna laxiflora* seeds and *Tectona grandis* bark has shown significant activity on comparison to the synthetic drugs Spironolactone, polyherbal formulation Cystone and to the other extracts of the drug, the furosemide was found to non-active on formed crystals (Mosquera, D. M. G., 2020).

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References

1. Asif, M. (2011). In vivo analgesic and antiinflammatory effects of *Tectona grandis* Linn. stem bark extracts. *Malaysian Journal of Pharmaceutical Sciences*, *9*(1), 1-11.
2. Bagali, R. S., Jalalpure, S. S., & Patil, S. S. (2020). *In-vitro* Antioxidant and *In-Vivo* Hepatoprotective Activity of Ethanol Extract of *Tectona grandis* Bark Against CCl4 Induced Liver Injury in Rats. *Pharmacognosy Journal*, *12*(3)., 598-602.
3. Ghaisas, M. M., Navghare, V. V., Takawale, A. R., Zope, V. S., & Deshpande, A. D. (2008). *In vitro* antioxidant activity of *Tectona grandis* Linn. *Pharmacologyonline*, *3*, 296-305.
4. Mishra, H., Rai, K., & Alex, A. (2022). *In vitro* antilithiatic and antioxidant potential of ethanolic extract of *Bauhinia variegata* leaves. *International Journal of Health Sciences*, *6*(S5), 3264–3280. https://doi.org/10.53730/ijhs.v6nS5.9354
5. Mosquera, D. M. G., Ortega, Y. H., Quero, P. C., Martínez, R. S., & Pieters, L. (2020). Antiurolithiatic activity of *Boldoa purpurascens* aqueous extract: An *In vitro* and in vivo study. *Journal of ethnopharmacology*, *253*, 112691.
6. Qazi Majaz, A., & Molvi Khurshid, I. (2015). A comprehensive review on meyna Laxiflora robyns (rubiaceae). *Int. J. Pharm. Sci. Rev. Res*, *35*, 22-25.
7. Rai, K., Mishra, H., & Alex, A. (2022). *In vitro* antilithiatic and antioxidant potential of methanolic extract of *Polyalthia longifolia* leaves. *International Journal of Health Sciences*, *6*(S6), 2582–2598. https://doi.org/10.53730/ijhs.v6nS6.10041
8. Salem, P. P., Vieira, N. B., Garcia, D. A., Nicácio, K. J., Dias, D. F., de Paula, A. C., ... & Chagas-Paula, D. A. (2020). Anti-urolithiatic and anti-inflammatory activities through a different mechanism of actions of Cissus gongylodes corroborated its ethnopharmacological historic. *Journal of ethnopharmacology*, *253*, 112655.
9. Siddiqui, W. A., Shahzad, M., Shabbir, A., & Ahmad, A. (2018). Evaluation of anti-urolithiatic and diuretic activities of watermelon (Citrullus lanatus) using in vivo and *In vitro* experiments. *Biomedicine & Pharmacotherapy*, *97*, 1212-1221.
10. Vyas, P., Wadhwani, B. D., Khandelwal, P., Araya, H., & Fujimoto, Y. (2022). Tectonaquinones A, B and C: three new naphthoquinone derivatives from the heartwood of *Tectona grandis*. *Natural Product Research*, *36*(7), 1707-1715.
11. Wang, R., Younis, E. M., Veeraraghavan, V. P., & Tian, C. (2020). Antiurolithiatic effect of Fucoxanthin on ethylene glycol-induced renal calculus in experimental rats. *Journal of King Saud University-Science*, *32*(3), 1896-1901.