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Evaluation of *in-vitro* Antiurolithiatic Potential of Ethanol, Hexane, and Aqueous Extracts of *Aegle marmelos*

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Background: Medicinal plants have been extensively studied for their various biological activities and therapeutic potentials including antiurolithiatic potential. Calcium oxalate (CaOx) is the predominant component of most stones that are deposited in the urinary system. Formation of these stones is a multi-step process which includes super saturation, nucleation, aggregation, growth and retention. *Aegle marmelos* is commonly used by the ayurvedic medical practitioners in the treatment of urolithiasis.

Objectives: To determine the effect of ethanol, hexane and aqueous extracts of *Aegle marmelos* (Beli mal) against CaOx urolithiasis *in-vitro*

Methods: Three extracts of *Aegle marmelos* were analyzed for *in-vitro* antiurolithiatic activity using nucleation, aggregation and growth assays. Optical density of reaction mixture was measured spectrophotometrically. Each test was carried out in duplicate. Type of crystals formed was observed under a light microscope. Preliminary phytochemical screening was performed.

Results: The results demonstrate that *Aegle marmelos* has the capacity to inhibit the nucleation, growth and aggregation of CaOx crystals. In nucleation assay, microscopic examination of crystals revealed the presence of more calcium oxalate monohydrate crystals than calcium oxalate dihydrate crystals. Also, a reduction of the number of crystals was observed in the presence of plant extracts compared to the control. Both ethanol and hexane extracts showed significantly higher inhibition compared to the standard drug, Cystone ($p \leq 0.05$) but the maximum percentage of inhibition was shown by hexane extract of *Aegle marmelos* at 1000 $\mu\text{g/mL}$ ($83.56 \pm 0.06\%$). In the growth and aggregation assays, all extracts showed significant inhibitions but aqueous extract of *Aegle marmelos* showed the maximum inhibition against CaOx crystal growth and aggregation, at 1000 $\mu\text{g/mL}$ (75.76%, 50.13% respectively). Aqueous extract revealed the presence of reducing sugars, proteins, anthracene glycosides and saponins.

Conclusions: This study has proven the *in-vitro* antiurolithiatic potential of *Aegle marmelos* against CaOx urolithiasis. Since maximum inhibitory activity was shown against both growth and aggregation of CaOx crystals, aqueous extract of *Aegle marmelos* has higher antiurolithiatic potential compared to other two extracts.

Keywords: *Aegle marmelos*, Calcium oxalate, Nucleation, Urolithiasis