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EVALUATION OF METHANOLIC EXTRACT OF *MACROTYLOMA UNIFLORUM* SEEDS ON INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION- *IN-VITRO*

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ABSTRACT: Seeds of *Macrotyloma uniflorum* Linn. (Fabaceae) are widely used for treatment in kidney stones in India. In This study evaluates the *in-vitro* effect of methanolic extract of *Macrotyloma uniflorum* (MEMU) on calcium oxalate crystallization. A methanolic extract of *M. uniflorum* seeds at different concentrations (250-1500 µg/ml) was subjected to *in-vitro* crystallization activity. The obtained results were compared with a Cystone (standard herbal product) available in the market. The nucleation and crystal growth assay was performed with different concentrations of sodium oxalate (2-10 mmol/ml for nucleation, 2-3.5 mmol/ml for crystal growth) and evaluated the effect of MEMU and cystone at different doses (250-1500 µg/ml) on the different concentration level of oxalate. Crystal aggregation and crystal dissolution assay were performed and compared with cystone effect. The MEMU was significantly more effective than cystone at inhibiting the nucleation rate and crystal growth at a different concentration level of oxalate. Inhibition of calcium oxalate crystal aggregation and dissolution was observed in a dose-dependent manner. The results showed that MEMU has excellent *in-vitro* calcium oxalate crystallization inhibition activity in the different concentration level of oxalate and also have crystal dissolution activity; therefore, it might be useful in the prevention of renal stones.

INTRODUCTION: Kidney stones are the solid crystalline masses that occur anywhere in the urinary system, and it is often correlated with habits such as high purine intakes, obesity, hypertension, metabolic syndrome, and diabetes [1]. A large number of people, nearly 4-15% of the human population, suffer from the renal stone problem all over the global [2].

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As per the epidemiological studies, men are more affected as compared to women and are more prevalent between 20-49 ages in both sexes [3]. The recurrence rate every year is 10-23%, in that 50% occurred in 5-10 years, and 75% occurred in 20 years.

Areas with a higher incidence of urolithiasis are Central Europe, Scandinavian countries, British Isles, Mediterranean countries, Northern Australia, portions of the Malayan Peninsula, China, Pakistan, and northern India. In India, Maharashtra, Gujarat, Punjab, Haryana, Rajasthan, and Delhi are high incidence states for renal stones. Approximately 12% population of India is expected to have renal stones, out of which 50% may end up with a loss of

kidneys or renal damage. Since, stone formation is a multi-factorial disease, with complex etiology is and highly unpredictable [4]. A majority of renal stones are composed of calcium, oxalate, uric acid, phosphate, struvite, and cystine. Amongst all of these calcium oxalate stones are the most common renal stone found in almost 80% cases where 5-10% uric acid stone is found to be present. There are two different types of calcium oxalate (CaC_2O_4) stones, calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD). COM is a thermodynamically more stable form than COD and has a greater affinity for renal tubular cells [5]. CaC_2O_4 stone formation is a biological process that involves physicochemical elements and crystal-ization, which includes supersaturation of ions in urine, nucleation, crystal growth, crystal aggregation, and crystal retention within the renal tubules.

The supersaturation of calcium and oxalate ions in urine results in spontaneously crystallized the particles and increased the nucleation formation in fluid6. After nucleation, free particles present in the fluid, attached to the preformed CaC_2O_4 crystal, and increase in the crystal size. Small crystals were easily excreted in the urine, but numerous crystal comes together which is promoted by viscous binding and adhere to form large crystal which usually retains in retain in renal tubules and promoting the stone formation [7].

Thus, crystal formation can be prevented by stopping the crystal retention by decreasing the nucleation, crystal growth, and crystal aggregation process in a fluid. At Present-day in the medical management of renal stone mainly involves the surgical removal of stones. Techniques such as percutaneous nephrolithotomy, extracorporeal shock wave lithotripsy, ureteroscopy, but these techniques do not assure the prevention of recurrence of the stone formation [8]. Hence, the search for herbomineral preparations is still required. A large number of Indian medicinal plants have been used in the treatment of urolithiasis, and they are reported to be effective without any side effects [9]. Horse gram (*Macrotyloma uniflorum* [Lam.] Verde.) is extensively cultivated, especially in dry areas of Australia, Burma, India, and Sri Lanka. The plant is used as a vegetable in India, and the seed is known as the poor man's pulse in southern India. Consumption of horse gram seeds, after soaking / dry heating followed by cooking, along with cooked rice or pearl millet, is common among the rural people in India [10]. Seeds of *M. uniflorum* contain varying amounts of carbohydrates, proteins, amino acids, lipids, phenolic acids, flavonoids, tannins, phytosterols, fatty acids, anthocyanidins, saponins and minerals like iron, calcium, and molybdenum. Phenolic acids obtained from *M. uniflorum* are considered to be potent antioxidants that act by scavenging free radicals and ROS [11].

According to Ayurveda, the seeds are bitter, acrid, dry, hot and used as astringent, anthelmintic, antipyretic and in conditions such as heart-troubles, uterine stones, asthma, tumors, bronchitis, hiccup, urinary discharges, diseases of the brain and eyes, intestinal colic, piles, inflammation, liver troubles, *etc*. Traditional healers recommend *M. uniflorum* seeds water infusion for kidney and gall stones patients [12]. However, so far, no scientific study of aqueous extract has been reported regarding the antiurolithiatic potential of the plant. Thus, the objective of this study is to evaluate the preventive and beneficial effects of methanolic extract of *M. uniflorum* on experimental calcium oxalate crystallization *via in-vitro* methods.

MATERIALS AND METHODS:

Materials: All chemicals used in the study were of analytical grade. Standard drug cystone (Himalaya Drug Company) purchased from the local market of Ahmedabad.

Plant Material and Preparation of Plant Extract: *M. uniflorum* seeds were purchased from the local market of Ahmedabad, Gujarat, India. Seeds were authenticated by ethnobotanist, Head, P.G. Centre in Botany, Smt. S. M. Panchal Science College, Talod, Gujarat, India. The specimen was submitted to the Pharmacognosy department of the Institute of Pharmacy, Nirma University. (Ref No. IPNSAVPMU2015). Dry seeds were grounded into the fine powdered by using an electric grinder. Powdered material was stored in the tight container at ambient temperature. The powder material of *M. uniflorum* (100 gm) was refluxed with 500 ml of methanol for 24 h. The methanol layer was filtered & evaporated using a rotary vacuum evaporator at

50 °C, which collected dry extract (7.45% w/w) was labeled as MEMU (Methanolic extract of *M. uniflorum*).

Phytochemical Screening and Quantitative Estimation of Phytoconstituents: Phytochemical screening of MEMU was carried out to identify the nature of phytoconstituents present in the extract. Total flavonoid content was measured by aluminum chloride colorimetric assay [13] and expressed as milligram of quercetin equivalent per gram of extract. Total saponins were determined according to the methods previously described by Obadoni and Ochuko [14].

***In-vitro* Experiments:** For the *in-vitro* evaluation of MEMU on calcium oxalate crystallization following solution was prepared. A solution of sodium oxalate (1-5 mmol/l) and calcium chloride (1-5 mmol/l) were prepared in a buffer containing Tris–HCl 0.05 mol/l and NaCl 0.15 mol/l at pH 6.5.

Nucleation Assay: Nucleation assay was performed with some modification in the method described by Hennequin *et al.,* [15] In this method 9 ml of calcium chloride (5 mmol/l) solution was mixed with 1 ml of MEMU at different concentration (250, 500, 750, 1000, 1250, 1500 µg/ml) and 9 ml of sodium oxalate at different concentration of ranging from (1-5 mmol/ml) were added in each beaker. The temperature was maintained at 37 °C. The optical density of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of MEMU with that of control. For standard drug (cystone), 1 ml of cystone at different concentrations (250, 500, 750, 1000, 1250, 1500 µg/ml) was added in replacement of MEMU. The percentage inhibition of extract and standard drug were calculated by

Percentage Inhibition = OD (Control) – (OD (exp)) / (OD (Control) × 100

Crystal Growth Assay: Newly formed crystals may combine to form a small hard mass, called calculus. The percentage inhibition of calcium oxalate crystal growth will be evaluated in the presence and absence of MEMU by adapting the procedure described by Chaudhary *et al.,* [16,] with some modification. 1 ml of calcium oxalate monohydrate crystal slurry was mixed with 10 ml of 2 mM calcium chloride solution immediately add 10 ml of sodium oxalate solution in different conc. (2, 2.5, 3, 3.5 mM). Consumption of oxalate immediately started after the addition of sodium oxalate solution, and the solution was magnetically stirred at 800 rpm for 15 min, and absorbance was recorded at 214 nm, with and without MEMU. The relative inhibitory activity was calculated as follows:

% relative inhibitory activity = ((C-S)/C) × 100

Where ‘C' is the rate of reduction of free oxalate without any extract and ‘S' is the rate of reduction of free oxalate with the extract.

Aggregation Assay: Aggregation assay has been performed using a method described by Atmani and Khan [17] with some modification. Calcium oxalate crystals seeds were prepared by mixing calcium chloride (50 mmol/l) and sodium oxalate (50 mmol/l). Both solutions were incubated for 1 hr at to 60 °C in a water bath and cooled at room temperature for overnight. The crystals were harvested by centrifugation and then evaporated at 37 °C. Calcium oxalate crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris–HCl 0.05 mol/l and NaCl 0.15 mol/l at pH 6.5. Experiments were conducted at 37 °C in the absence or presence of the MEMU. The percentage inhibition of aggregation was calculated by comparing the turbidity in the presence of MEMU at different concentrations (250, 500, 750, 1000, 1250, 1500 µg/ml) with that obtained in control using the following formula:

% Inhibition of aggregation= 100 – Rate of aggregation (IR)

Where IR = (Turbidity of sample / Turbidity of control) × 100

Calcium Oxalate Dissolution: Calcium oxalate crystal dissolution assay performs as described by saso *et al.,* [18], followed by some modification. In that calcium oxalate seeds were prepared as per mention in aggregation method, each seed weight approximately 10 mg. After that 1 ml of MEMU (250, 500, 750, 1000, 1250, 1500 µg/ml) solution at pH 6 were added to the 10 mg of calcium oxalate seed in tubes followed by incubation overnight at room temperature under mild mixing by a vortex. Then the tubes were centrifuged, and seeds washed,

dried, and weighed again. The ability of AEMU to dissolve calcium oxalate seed was calculated with the following formula

% Crystal dissolution = ((Initial weight – final weight)/Initial weight) × 100

Statistical Analysis: The results are expressed as mean ± SEM. Statistical analysis and linear regression analysis were performed using GraphPad Instat, software, version 3.0.

RESULTS:

Phytochemical Screening and Quantitative Estimation of Phytoconstituents: The MEMU was subjected to a qualitative analysis of the various phytoconstituents using chemical tests. The study revealed the presence of carbohydrates, Protein, glycosides, alkaloids, saponin, flavonoid, tannins, and phenolic compounds. The total flavonoid content of MEMU was found to be 8.8 ± 0.32 mg quercetin equivalents/g of extract. The total saponin content of the powdered drug was found to be 29.44 ± 0.71 mg diosgenin equivalents/g of powder.

***In-vitro* Assay:**

Nucleation Assay: As nucleation formation starts in the solution, it becomes turbid, which estimated as the turbidity level of the solution. In the control group as the concentration of sodium oxalate increases (2, 4, 6, 8. 10 mM), nucleation formation increased. These nucleation formations were decreased in the presence of MEMU in a concentration-dependent manner.

The extract shows the highest inhibition (83.14 ± 0.44) at a higher dose (1500 µg/ml) in a lower concentration of sodium oxalate (2 mM), but as sodium oxalate level increase (4, 6, 8, 10 mM) inhibition activity was decreased (75.18 ± 0.28, 66.92 ± 0.21, 60.38 ± 0.24, 58.43 ± 0.16) at higher dose.

Table 1 while cystone showed 61.56 ± 0.34, 56.95 ± 0.17, 50.00 ± 0.17, 45.59 ± 0.12 and 41.48 ± 0.13 inhibition at higher concentration (1500 µg/ml) which was lower as compared to MEMU at 2, 4, 6, 8, 10 mM concentration of sodium oxalate respectively **Table 2**.

TABLE 1: EFFECT OF MEMU ON CALCIUM NUCLEATION WITH INCREASING CONCENTRATION OF SODIUM OXALATE (NAOX)

Concentration (µg/ml)	2 mM NaOx	4 mM NaOx	6 mM NaOx	8 mM NaOx	10 mM NaOx
250	40.48 ± 0.56	40.38 ± 0.35	39.19 ± 0.31	37.26 ± 0.26	35.85 ± 0.23
500	53.76 ± 0.67	49.96 ± 0.46	41.76 ± 0.30	41.62 ± 0.25	40.51 ± 0.26
750	61.04 ± 0.66	55.95 ± 0.35	49.84 ± 0.32	45.98 ± 0.27	43.39 ± 0.23
1000	64.11 ± 0.67	60.94 ± 0.34	55.85 ± 0.22	51.19 ± 0.22	50.70 ± 0.16
1250	75.35 ± 0.55	69.19 ± 0.29	62.77 ± 0.23	56.19 ± 0.07	54.93 ± 0.07
1500	83.14 ± 0.44	75.18 ± 0.28	66.92 ± 0.21	60.38 ± 0.24	58.43 ± 0.16

TABLE 2: EFFECT OF CYSTONE ON CALCIUM NUCLEATION WITH INCREASING CONCENTRATION OF SODIUM OXALATE (NAOX)

	% Inhibition of Nucleation				
Conc. of Drug (µg/ml)	2 mmol NaOx	4 mmol NaOx	6 mmol NaOx	8 mmol NaOx	10 mmol NaOx
250	37.93 ± 0.44	35.86 ± 0.17	32.78 ± 0.13	29.70 ± 0.18	25.22 ± 0.04
500	43.93 ± 0.56	42.31 ± 0.22	39.19 ± 0.17	34.91± 0.11	30.97 ± 0.13
750	50.96 ± 0.44	47.24 ± 0.17	43.33 ± 0.08	39.61 ± 0.07	34.44 ± 0.10
1000	54.79 ± 0.45	51.96 ± 0.18	46.82 ± 0.17	42.18 ± 0.15	37.86 ± 0.11
1250	58.62 ± 0.40	54.49 ± 0.23	48.63 ± 0.19	44.27 ± 0.11	40.46 ± 0.13
1500	61.56 ± 0.34	56.95 ± 0.17	50.00 ± 0.17	45.59 ± 0.12	41.48 ± 0.13

Crystal Growth Assay: After nucleation formation, another step in urolithiasis is crystal growth. As the oxalate concentration was increased, the crystal formation rate was also increased. In the present study, the prevention of crystal growth was directly related to the concentration of MEMU; the highest inhibition (82.40 ± 0.32) was observed at 1500 µg/ml. When we have increased the concentration of NaOx (2 to 3.5 mmol/ml), then at the same dose level, inhibition effect was decreased from 82.40 ± 0.32 to 55.80 ± 0.31. **Table 3** At a similar dose level, cystone showed similar inhibition in crystal growth, 73.10 ± 0.42 inhibition at 2 mM concentration of NaOx, and 44.23 ± 0.60 inhibition at 3.5 mM concentration of NaOx **Table 4**.

TABLE 3: EFFECT OF MEMU ON CALCIUM OXALATE CRYSTAL GROWTH AS AN INCREASE IN SODIUM OXALATE LEVEL

	% Inhibition of crystal growth			
Conc. of Drug (µg/ml)	**2 mmol NaOx**	**2.5 mmol NaOx**	**3 mmol NaOx**	**3.5 mmol NaOx**
250	66.01 ± 0.24	56.18 ± 0.23	45.03 ± 0.39	42.48 ± 0.46
500	67.43 ± 0.31	59.36 ± 0.32	47.88 ± 0.24	45.68 ± 0.27
750	72.17 ± 0.41	60.76 ± 0.31	49.92 ± 0.17	48.10 ± 0.11
1000	75.37 ± 0.32	64.50 ± 0.48	53.44 ± 0.22	49.47 ± 0.10
1250	79.39 ± 0.35	67.08 ± 0.25	55.30 ± 0.53	53.23 ± 0.30
1500	82.40 ± 0.32	70.04 ± 0.13	57.96 ± 0.24	55.80 ± 0.31

TABLE 4: EFFECT OF CYSTONE ON CALCIUM OXALATE CRYSTAL GROWTH AS AN INCREASE IN SODIUM OXALATE LEVEL

	% Inhibition of crystal growth			
Conc. of Drug (µg/ml)	**2 mmol NaOx**	**2.5 mmol NaOx**	**3 mmol NaOx**	**3.5 mmol NaOx**
250	54.93 ± 0.49	51.15 ± 0.39	31.76 ± 0.38	29.09 ± 0.42
500	58.92 ± 0.42	54.21 ± 0.45	36.53 ± 0.39	31.18 ± 0.40
750	62.83 ± 0.41	58.16 ± 0.46	42.35 ± 0.45	35.13 ± 0.41
1000	67.23 ± 0.40	60.85 ± 0.51	45.11 ± 0.45	38.26 ± 0.48
1250	69.43 ± 0.56	65.02 ± 0.32	49.14 ± 0.58	41.11± 0.30
1500	73.10 ± 0.42	68.45 ± 0.22	51.60 ± 0.53	44.23 ± 0.60

Aggregation Assay: In aggregation assay, calcium oxalate crystals were less aggregated in the presence of MEMU in a concentration-dependent manner. Cystone also showed the inhibition of aggregation; however, it was less effective as compared to the MEMU in the same concentration range, **Fig. 1**.

The MEMU showed 34-55% inhibition with IC_{50} = 1327.86 µg/ml, where cystone showed 32-45% inhibition with IC_{50} = 1816.36 µg/ml **Table 5**.

TABLE 5: EFFECT OF MEMU AND CYSTONE ON CRYSTAL AGGREGATION

	% Inhibition of crystal aggregation	
Conc. of Drug (µg/ml)	**MEMU**	**Cystone**
250	33.88 ± 0.20	32.51 ± 0.14
500	36.68 ± 0.29	36.06 ± 0.25
750	44.16 ± 0.20	39.12 ± 0.19
1000	47.52 ± 0.27	42.02 ± 0.23
1250	50.68 ± 0.31	44.10 ± 0.19
1500	53.08 ± 0.24	47.32 ± 0.23

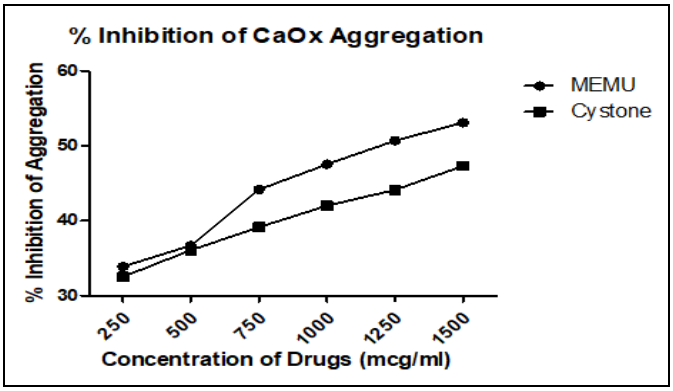

FIG. 1: EFFECT OF MEMU AND CYSTONE ON CRYSTAL AGGREGATION

Calcium Oxalate dissolution: In Calcium oxalate dissolution assay, after incubation of overnight CaOx seeds with mild vortexed mixing, the weight of CaOX seeds was decreased as the concentration of drug was increased. At lower concentration (250 µg/ml) of MEMU showed 37.56% calcium oxalate seeds dissolution as compared to the cystone drug which showed 44% dissolution which was higher as compared to MEMU but when increase at higher concentration (1500 µg/ml) of MEMU showed the 66.20% dissolution which was higher as compared to cystone which showed the 59.86% dissolution at the same dose **Fig. 2** and **Table 6**.

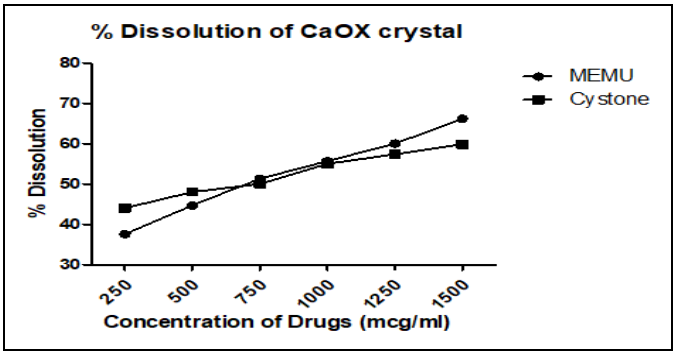

FIG. 2: EFFECT OF MEMU AND CYSTONE ON CAOX CRYSTAL DISSOLUTION

TABLE 6: EFFECT OF MEMU AND CYSTONE ON CRYSTAL DISSOLUTION

	% Dissolution of CaOx crystals	
Conc. of Drug (µg/ml)	**MEMU**	**Cystone**
250	37.56 ± 0.58	44.00 ± 0.76
500	44.66 ± 0.60	48.00 ± 0.28
750	51.33 ± 0.72	50.00 ± 0.29
1000	55.66 ± 0.44	55.00 ± 0.86
1250	60.00 ± 0.28	57.40 ± 0.49
1500	66.20 ± 0.72	59.86 ± 0.41

DISCUSSION: Stone formation is a biological process that involves a physicochemical element and crystallization. An important factor in crystallization is nucleation that leads to crystal growth and crystal aggregation, which is responsible for stone formation. When urine was supersaturated with stone materials that indicate the ions concentration is higher than its thermodynamic solubility due to that crystal formation begins in the urine. Nuclei of the crystal cannot grow large without attaching to the renal tubules and enter into the renal pelvis. Large crystals aggregate into large clumps within a few minutes and convert into the large crystal, which finally retains into the urinary tract [19]. Retention of crystal in renal induced the renal tubular cell injury, which provides the suitable environment for the generation of new nuclei of calcium oxalate on the renal papillary surface and further support crystal nucleation at lower supersaturation level [20]. Agents that cause inhibition of crystallization and modifiers of this processor decreased oxalate supersaturation are major interest agents for urolithiasis treatment. Various inhibitors can affect crystal nucleation, growth, or aggregation [21]. The organic compounds adsorb to the surface of a crystal, thereby inhibiting crystal nucleation, growth, and aggregation [7].

Many calcium oxalate inhibitors available in urine like glycosaminoglycans [22], magnesium, and citrate [23], but these macromolecules are not increased in urine because of their high molecular weight and limited use of these inhibitors as a clinically. It has been reported that sodium dodecyl sulphate, metallic ions, and their complexes, maleic acid copolymers, and a protein from the human kidney showed *in-vitro* anti-crystallization property, but their clinical use is limited [24]. In traditional medicine, anti-lithogenic properties can be exhibited either by increasing the urine volume and pH or by balancing the process of inhibition and promotion of the crystallization in urine as it affects the crystal nucleation, growth, and aggregation [25].

Many phytoconstituents are present in herbal medicines which give beneficial effects by relieving the binding mucin of calculi or by regulation of the crystalloid-colloid imbalance [26]. In this present study, the *in-vitro* inhibitory effect of aqueous extract of *M. uniflorum* on calcium oxalate crystallization.

As discussed earlier urinary supersaturation correlate with crystal formation, as the oxalate level increase in urine, nucleation formation rate also increases and lowering the supersaturation or oxalate levels is more effective for preventing crystallization or nucleation. In the present study, seed extract inhibits the nucleation formation in a dose-dependent manner concerning the concentration level of sodium oxalate. This activity of MEMU could be its ability to complex with oxalate and calcium ions in solution and decrease supersaturation level. However, nucleation formation is a common process in healthy and urolithiasis patients, in healthy patient small nuclei easily excreted in the urine, but in urolithiasis patient after nucleation, free calcium and oxalate particles which present in urine, attached to the preformed calcium oxalate crystal and increase in the crystal size.

Crystal growth was dependent on the concentration level of oxalate and calcium. The present study showed that MEMU prevents crystal growth in a dose-dependent manner. Still, the inhibition rate was found deceased at the same dose of an extract with the gradual increase in sodium oxalate concentration. Small crystals were easily excreted in the urine, but numerous crystals come together and adhere to form large crystals which usually retains in renal tubules and promoting the stone formation. Therefore, the crystal aggregation process is thought to be a fundamental step in renal stone development. MEMU inhibited the *in-vitro* calcium oxalate aggregation in a concentration-dependent manner. Some findings suggested that chemical treatments may be useful for improving the efficacy of stone treatment via dissolving larger or harder stones. Zhou *et al.*, [27] reported that buffered EDTA solvents might be feasible chemical treatment modalities for improving the efficacy of calcium oxalate stone dissolution.

In the present study, MEMU was found active in dissolving the calcium oxalate stones in a concentration-dependent manner, which indicates that MEMU contains some of the components that constitute to increase the calcium oxalate stone dissolution. Qualitative phytochemical estimation of MEMU revealed the presence of flavonoids, saponins, and phenolic compounds. These phytoconstituents are of utmost significance for

inhibiting urinary stone formation. Saponins possess antilithic properties and are known to disintegrate mucoproteins that are crucial components of the stone matrix [27]. Rutin, quercetin, hyperoside, and diosmin are known as flavonoids with high antioxidant and antilithiatic activities [28]. MEMU contains 8.8 ± 0.32 mg quercetin equivalents/g flavonoids and 29.44 ± 0.71 mg diosgenin equivalent/g saponin. Therefore, the prevention of nucleation, crystal growth, crystal aggregation, and Crystal dissolving property of MEMU.

Cystone, a polyherbal market formulation used in the treatment of kidney stones. Which contains extracts of Hajrul yahood bhasma, *Didymocarpus pedicellata*, *Rubia cordifolia*, *Cyperus scariosus*, *Onosma bracteatum*, *Achyranthes aspera*, *Saxifraga ligulata* and *Veronica cincerea*. Cystone inhibit the calcium oxalate crystallization, via inhibiting the initial precipitation of calcium and phosphate ions in the form of mineral phase bound to the organic matrix and the subsequent growth of the preformed mineral phase. Thus, the MEMU might contain compounds that could stimulate the demineralization of the matrix-bound mineral phase.

CONCLUSION: It can be concluded that methanolic extract of *M. uniflorum* was able to prevent nucleation, crystal growth, crystal aggregation, and crystal dissolution *in-vitro*. It can be confirmed that MEMU has antilithiatic property, which might be due to the presence of flavonoids, saponins, and other active components. But as we know, *in-vitro* studies have many limitations, when *in-vitro* active extract or substance administered orally or intravenous to living systems, the active substance modifies to various forms before they act on the specific target. So, to understand the specific activity and mechanism of MEMU, further *in-vivo* studies required.

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CONFLICTS OF INTEREST: Nil

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