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In vitro Anti-Urolithiatic Activity of Aqueous Extract of *Macrotyloma uniflorum*

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A B S T R A C T

Objective: Urolithiasis is one of the oldest and most widespread urinary system diseases affecting millions globally. Herbal treatment is necessary to prevent and cure stone formation because of the high incidence and recurrence rate of stone formation, which is linked to significant discomfort and inflammation in the urinary system. Urolithiasis is treated with a variety of herbal preparations in India, which are renowned for their undeniable safety and effectiveness. In India, *Macrotyloma uniflorum* seeds are frequently used to treat kidney stones. The present study evaluates *in vitro* effect of aqueous extract of *M. uniflorum* (AEMU) on calcium oxalate crystallization.

Methods: Different concentrations of an AEMU (250–1500 µg/mL) were tested for in vitro activity. The outcomes were contrasted with those of a common herbal product that is sold on the market, Cystone. A fixed concentration of calcium chloride was incubated with different concentrations of sodium oxalate (2–10 mmol/mL) for nucleation and (2–3.5 mmol/mL) for crystal growth in order to perform the nucleation and crystal growth assay and assess the inhibitory effect of AEMU at various oxalate concentration levels. Assays for the aggregation and dissolution of calcium oxalate crystals were conducted and the results compared to the standard effects. Every step was carried out three times, and the analysis was done in Microsoft Excel to determine the percentage inhibition and dissolution in comparison to a standard control Cystone.

Results: When tested at various sodium oxalate concentrations, the results demonstrated that AEMU possesses exceptional calcium oxalate crystallization inhibitory activity in nucleation rate, crystal development, crystal aggregation and increased crystal dissolution. Compared to the herbal medication Cystone, a greater effect was seen in the dose-dependent manner in AEMU.

Conclusion: These in vitro results offer convincing proof of AEMU's potent crystallization inhibition and crystal dissolving capabilities.

Keywords: *Macrotyloma uniflorum*, Urolithiasis, Calcium Oxalate Stones

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Introduction

Urolithiasis, also known as renal stone disease, is the painful third urinary ailment. Around 4800 BCE, it was first discovered in Egyptian mummies. It may result in renal calcification and has a significant effect on public health [1–3]. Urinary stones affect 4 to 15% of the global population. A recent study found that between 1990 and 2019, the incidence of urolithiasis rose by 48.57% globally. [4] According to the 2012 National Health and Nutrition Examination survey, 10.6% of men and 7.1% of women in the US had renal kidney stones [5]. According to epidemiological research, men between the ages of 20 and 49 are more likely to have the ailment. Recurrence rates range from 10% to 23% annually, with 50% of cases occurring within 5–10 years and 75% within 20 years. It is estimated that 12% of Indians suffer from urolithiasis annually, with the largest incidence rates occurring in the "Stone Belt" states of Rajasthan, Gujarat, Delhi, Maharashtra, Haryana, and Punjab [6] .

The etiology of urolithiasis is complex and very varied due to its multifaceted character. Renal stones are caused by a variety of endogenous and exogenous variables in addition to multivariate pathophysiology. There are two forms of calcium oxalate crystals, COM- Calcium oxalate monohydrate and COD- Calcium oxalate dehydrate. Compared to COM, COD is less thermodynamically stable and exhibits a lower affinity for renal tubular cells [7]. The biological process of CaC2O4 stone

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production includes physicochemical components and the formation of crystals, which includes the supersaturation of ions in urine, which initiate crystal nucleation process, followed by crystal growth. Accumulation of crystal called as crystal aggregation, which retain in the renal tissue called as crystal retention. [8] Urine's supersaturation of calcium and oxalate ions causes the particles to crystallize spontaneously and enhances fluid nucleation generation [9]. Following nucleation, loose liquid ions cling to the CaC2O4 crystals that had already formed and grown larger. The tiny crystals were easily removed from the urine, but large crystals are typically retained in the renal tubules and contribute to the formation of stones when many crystals join together and adhere through viscous binding [10]. Therefore, it is possible to stop the production of crystals by accelerating the dissolution of crystals and reducing their nucleation, development, and aggregation in fluid.

The management of urolithiasis is mostly determined by the location and size of the renal stones. In Majority of cases, stones are removed surgically using techniques like Urereroscopy, percutaneous nephrolithotomy, and extracorporeal shock wave lithotripsy; however, about half of these cases have a recurrence of the stone after removal. [11]. Aeckart and Schroder list kidney fibrosis, bleeding, tubular necrosis, and hypertension as adverse outcomes of surgical treatment [12]. Ayurveda suggests using a variety of herbal plants to treat urolithiasis. Herbal plants possess a multitude of advantageous properties, such as litholytic, analgesic, antibacterial, anti-inflammatory, and antispasmodic properties, without posing any adverse consequences [13].

Macrotyloma uniflorum [Lam.] verde, also referred to as horse gram, is widely grown throughout Australia, Burma, India, and Sri Lanka. Known by several names in India, including Ullavallu (Telugu), Kulattha (Sanskrit), Kollu (Tamil), Gahot (Kumaon and Garhwal), Kurti-kalai (Bengali), and Muthira (Malayalam), Gahot meaning "which initially breaks down stone" [14]. One of India's most nutrient-dense vegetable pulse crops, it has traditional therapeutic uses. In Southern India, horse gram seeds are known as "poor man's pulse". India's rural population often eats horse gram seeds and cooked rice together. The seeds are described as hot, dry, caustic, and bitter in Ayurveda. Historically, its decoction has been used to treat menstrual disorders and leucorrhea. Indian traditional medicine uses horse gram seeds to treat urinary stones, worms, hiccups, corpulence, and calculus diseases [15]. They are used to cure piles and urinary tract infections, and they also have astringent and tonic properties [16]. Furthermore, it is believed that the cooked liquor of M. uniflorum seeds mixed with spices generates heat and can be used as a remedy for fever, colds, and throat infections [17].

Carbohydrates, phenolic acids (p-coumaric acid, kaempferol, quercetin, and myricetin), Amino acids, lipids, Proteins, anthocyanidins (like delphinidin, malvidin, cyanidin, and petudin), phytosterols (stigmasterol and β-sitosterol), flavonoids (quercetin, kaempferol, and myricetin), saponins, fatty acids (hexadecanoic acid, hexanoic acid), tannins, and minerals (Fe, Ca, and Mo) are all present in the seeds of M. uniflorum. It is believed that M. uniflorum's phenolic acids, which scavenge ROS and free radicals, are the most potent antioxidants [18]. According to Ayurveda, the seeds are hot, dry, bitter, and acrid. They are also used as an anthelmintic, antipyretic, and astringent, and they are used to treat a variety of ailments, including liver problems, piles, inflammation, urinary calculus, asthma, cancers, bronchitis, and urinary discharges [19]. For patients, who suffering from kidney and gallstones, traditional healers suggest infusing of water with M. uniflorum seeds. Although M. uniflorum seed extracts have been used traditionally, modern scientific data remain scarce Therefore; we hypothesize that an aqueous extract of M. uniflorum seeds can inhibit calcium oxalate crystallization Materials and Methods

Materials

For the study purpose, we have used the Sodium oxalate, calcium chloride and Tris Buffer procured from Suvidhinath Laboratories, Baroda. Cystone (Himalaya Drug Company) was bought from Ahmedabad's local market.

Plant Material and Preparation of Plant Extract

The seeds of Macrotyloma uniflorum were acquired from the Ahmedabad, Gujarat, local market. The seeds were verified by the Head of the P.G. Center for Botany at Smt. S. M. Panchal Science College, Talod, Gujarat, India, who is also an ethnobotanist. The specimen was handed over to Nirma University's Institute of Pharmacy's Pharmacognosy department (IPNSAVPMU2015). An electric grinder was used to finely grind the dry seeds into a powder. For later use, the powder was kept at room temperature in a sealed container. 500 milliliters of distilled water were refluxed with 100 grams of powdered of M. uniflorum seeds for 24 hrs at evaporating temperature of water. An aqueous layer was filterered and a rotary vacuum evaporator operating at 50 °C was used to evaporate the aqueous layer. The resulting dry extract (8% w/w, 8 gm extract obtain from 100 gm extract) was then collected and designated as aqueous extract of M. uniflorum (AEMU).

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% Yield of extract = (obtained gram extract/total gram of powder) * 100

Phytochemical screening and quantitative estimation of phytoconstitutents

To determine the kind of phytoconstituents contained in the extract, AEMU underwent phytochemical screening. The total flavonoid content of the extract was determined using the aluminum chloride assay method, and the result was expressed as milligrams of quercetin equivalent per gram of extract [20]. The whole saponin content of the extract was measured using the procedure outlined by Obadoni and Ochuko [21].

In vitro experiments

Several experiments were used to evaluate the effects of AEMU on calcium oxalate crystallization in vitro. Solutions of sodium oxalate (1–5 mmol/L) and calcium chloride (1–5 mmol/L) were made in a buffer containing NaCl 0.15 mol/L and Tris–HCl 0.05 mol/L at pH 6.5 for order to conduct the in vitro test. In all assay control consider as a negative control.

Nucleation assay

The nucleation assay was done with some modifications to the method given by Hennequin et al. [22] In this process, 9 mL of sodium oxalate (1-5 mmol/L) was added to each beaker, along with 1 mL of AEMU (250 - 1500 µg/mL) and 9 mL of calcium chloride (5 mmol/L). For the conventional drug experiment, instead of AEMU, 1 mL of a cystone solution with different doses (250 - 1500 µg/mL) was added. A steady temperature of 37 degrees Celsius was maintained. At 620 nm, the optical density (OD) of the solution was measured, and formula 1 was used to estimate the nucleation rate.

$$\%\ Inhibition\ of\ Nucleation = \left(1 - \frac{OD\ (Sample)}{OD\ (Control)}\right) * 100 \quad \text{---------- (1)}$$

COM crystal Preparation

The preparation of COM crystal seeds involved combining solutions of NaOx (50 mmol/L) and CaCl2 (50 mmol/L). The mixture was then incubated for one hour at 60 °C in a water bath, cooled to room temperature, and stored for the night. Centrifugation was used to extract the calcium oxalate crystals, which were subsequently evaporated at 37 °C.

Crystal growth assay

Calculus is a tiny hard mass that can be generated by the combination of newly formed crystals. The crystal growth assay, which assessed crystal growth in the presence and absence of AEMU, was carried out with some modifications to the method outlined by Chaudhary et al [23]. 10 mL of a 2 mM calcium chloride solution was combined with 1 mL of COM crystal slurry. Incorporate 10 milliliters of sodium oxalate solution at varying doses (2 -3.5 mM) right away. Following the addition of sodium oxalate, oxalate consumption began right away. The solution was shaken for 15 minutes at 800 rpm with a magnetic stirrer, both with and without AEMU, and the absorbance was measured at 214 nm using standard drug cystone solution at various concentrations (250, 500, 750, 1000, and 1250 µg/mL). Using equation 2, the relative inhibitory activity was determined.

$$\%\ Relative\ Inhibitiory\ Activity = \left(\frac{C-S}{C}\right) * 100 \quad \text{---------- (2)}$$

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

The Atmani and Khan method was used to carry out the aggregation assay [24]. The calcium oxalate crystals were made using the process described above, and they were then used at a concentration of 0.8 mg/mL in a buffered solution of Tris-HCl 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37°C with and without the AEMU and cystone. Formula 3 was used to calculate the percentage inhibition of aggregation by comparing turbidity in the presence of AEMU and cystone at different concentrations (250- 1250 µg/mL) to the control.

$$\%\ Inhibition\ of\ aggregation = 100 - Rate\ of\ aggregation\ (IR) \quad \text{---------- (3)}$$

Where, rate of aggregation is defined using equation 4

$$Rate\ of\ aggregation\ (IR) = \frac{Turbidity\ of\ sample}{Turbidity\ of\ control} * 100 \quad \text{---------- (4)}$$

Calcium oxalate dissolution

The calcium oxalate crystal dissolution assay was carried out mostly according to Saso et al.'s instructions, with a few minor adjustments. The crystal growth process described above was followed in order to prepare the seeds of calcium oxalate [25]. A series of AEMU solutions ranging in concentration from 250

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µg/mL to 1500 µg/mL were created. One milliliter of AEMU solution was added to ten milligrams of calcium oxalate seed in tubes, and the mixture was gently mixed with a vortex mixer. The mixture was then allowed to sit at room temperature for the entire night. The seeds were cleaned, dried, and weighed one more after the tubes were centrifuged. Using formula 5, the capacity of AEMU to dissolve calcium oxalate seeds was computed.

$$\%\ Crystal\ Dissolution = \frac{Inital\ Weight - Final\ Weight}{Intial\ Weight} * 100 \text{ ---------- (5)}$$

Statistical analysis

All assays were performed in triplicate (n=3). Results data were expressed as mean ± SEM. Statistical analysis was performed using Graphpad Prism version 6 and MS Excel. Statistical comparison used in comparison with percentage inhibition in compares with negative control (100 % growth) or dissolution and compare with standard Cystone drug.

Results

Phytochemical screening and quantitative estimation of phytoconstituents

Chemical assays were used to qualitatively analyze the different phytoconstituents in the AEMU. Carbohydrate, alkaloids, protein, glycosides, flavonoids, phenolics, tannins, and saponin, constituents were found in the study. It was discovered that AEMU has a total flavonoid concentration of 4.72 ± 0.12 mg quercetin equivalents/g of extract. The medication in powder form has a total saponin content of 36.44 ± 0.78 mg diosgenin equivalents/g.

In vitro study

Nucleation assay

The solution turns unclear as nucleation formation begins, and this is determined as the solution's turbidity level. Nucleation formation increased in the control group when sodium oxalate concentration increased (2, 4, 6, 8, 10 mM). In the presence of AEMU, these nucleation forms diminished in a concentration-dependent way. The extract exhibits the highest level of inhibition (80.46 ± 0.12) at higher dose (1500 µg/mL) when sodium oxalate concentration is lower (2 mM). However, when sodium oxalate levels increase to 4 mM, the AEMU inhibitory activity decreases from 80.46 ± 0.12 to 62.28 ± 0.11 at higher dose (1500 µg/mL), as table 1 illustrates. Cystone showed 61.56 ± 0.34 and 56.95 ± 0.17, inhibition at higher concentration (1500 µg/mL) which was lower as compared to AEMU at 2 mM and 4mM concentration of sodium oxalate respectively, as shown in table 2.

Table 1: Effect of AEMU on calcium nucleation with the increasing amount of sodium oxalate

Concentration of Drug (µg/mL)	% Inhibition of Nucleation				
	2 mmol NaOx	4 mmol NaOx	6 mmol NaOx	8 mmol NaOx	10 mmol NaOx
250	45.97 ± 0.22	43.91 ± 0.12	40.15 ± 0.08	39.74 ± 0.07	36.03 ± 0.04
500	55.55 ± 0.24	50.23 ± 0.18	42.52 ± 0.13	41.55 ± 0.08	38.17 ± 0.06
750	58.11 ± 0.33	53.22 ± 0.17	44.85 ± 0.08	43.84 ± 0.07	39.65 ± 0.07
1000	63.47 ± 0.35	55.82 ± 0.15	47.07 ± 0.13	46.28 ± 0.06	41.82 ± 0.06
1250	73.43 ± 0.34	59.88 ± 0.13	50.25 ± 0.12	48.59 ± 0.07	43.85 ± 0.10
1500	80.46 ± 0.22	62.28 ± 0.12	53.33 ± 0.09	51.15 ± 0.05	45.25 ± 0.07

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Table 2: Effect of cystone on calcium nucleation with increasing amount of sodium oxalate

Concentration of Drug (µg/mL)	% Inhibition of Nucleation				
	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, crystal development is a further stage in urolithiasis. The rate of crystal formation increased in tandem with a rise in oxalate content. The current investigation revealed a direct correlation between the prevention of crystal development and the concentration of AEMU, with the maximum inhibition (73.83 ± 0.17) recorded at 1500 µg/mL. Table 3 indicates that the inhibition rate fell from 73.83 ± 0.17 to 50.00 ± 0.08 when the quantity of sodium oxalate was increased from 2 mM to 3.5 mM at the same dose (1500 µg/mL). Table 4 illustrates that cystone exhibited comparable inhibition in crystal development at comparable dose levels (1500 µg/mL), with 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 3: Effect of AEMU on calcium oxalate crystal growth with the increasing amount of sodium oxalate

Concentration of Drug (µg/mL)	% CaOx crystal growth			
	2 mmol NaOx	2.5 mmol NaOx	3 mmol NaOx	3.5 mmol NaOx
250	57.94 ± 0.14	53.24 ± 0.13	38.47 ± 0.13	34.37 ± 0.12
500	61.36 ± 0.12	55.48 ± 0.12	43.17 ± 0.12	37.29 ± 0.11
750	64.05 ± 0.09	59.73 ± 0.15	46.75 ± 0.11	40.00 ± 0.09
1000	67.23 ± 0.18	62.86 ± 0.09	50.78 ± 0.14	43.95 ± 0.08
1250	70.08 ± 0.21	67.56 ± 0.16	54.13 ± 0.14	45.83 ± 0.13
1500	73.83 ± 0.17	70.69 ± 0.10	57.71 ± 0.12	50.00 ± 0.08

Table 4: Effect of cystone on calcium oxalate crystal growth with the increasing amount of sodium oxalate

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Concentration of Drug (µg/mL)	% CaOx crystal growth			
	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, fig 1 and table 5 showed that calcium oxalate crystals were less aggregated in the presence of AEMU in concentration dependent manner. Cystone likewise demonstrated aggregation inhibition, however in the same concentration range, it was less successful than AEMU. With an IC50 of 1258 µg/mL, the AEMU demonstrated 33–54% inhibition, whereas the cystone demonstrated 32–45% inhibition with an IC50 of 1816.36 µg/mLl.

Table 5: Effect of AEMU and cystone on crystal aggregation

Concentration of Drug (µg/mL)	% Inhibition of crystal aggregation	
	AEMU	Cystone
250	33.61±0.09	32.51±0.14
500	37.48±0.14	36.06±0.25
750	45.25±0.09	39.12±0.19
1000	48.03±0.09	42.02±0.23
1250	51.80±0.10	44.10±0.19
1500	54.20±0.14	47.32±0.23

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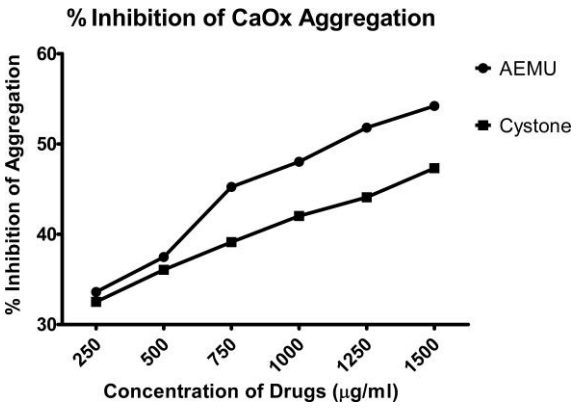

Figure 1: Effect of AEMU and cystone on in vitro CaOx aggregation

Calcium oxalate dissolution

In the calcium oxalate dissolving assay, the weight of the calcium oxalate seeds decreased as the drug concentration increased following an overnight incubation period with either AEMU or cystone solution under gentle vortexed mixing conditions. In comparison to the cystone medication, which showed 44% dissolving, AEMU at a lesser dose (250 µg/mL) showed 49.83% calcium oxalate seed dissolution. When AEMU was at a higher concentration (1500 µg/mL), its dissolution rate was 65.56%, which was greater than cystone's 50.96% dissolution at the same dose. (Figure 2 and Table 6)

Table 6: Effect of AEMU and cystone on crystal dissolution

Concentration of Drug (µg/mL)	% Dissolution of CaOx crystals	
	AEMU	Cystone
250	49.83 ± 0.44	44.00 ± 0.76
500	52.50 ± 0.28	48.00 ± 0.28
750	55.00 ± 0.28	50.00 ± 0.29
1000	57.50 ± 0.31	55.00 ± 0.86
1250	60.00 ± 0.25	57.40 ± 0.49
1500	65.00 ± 0.63	59.86 ± 0.41

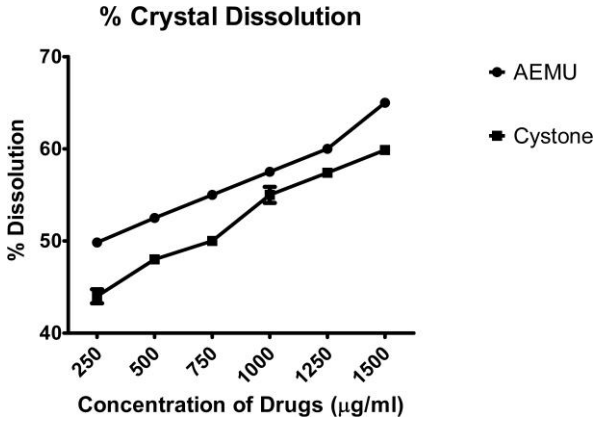

Figure 2: Effect of AEMU and cystone on CaOx crystal dissolution

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Discussion

Stone formation is one type of biological process that includes numerous physicochemical components and crystallization. Nucleation, which causes crystal growth and aggregation and is ultimately responsible for additional stone creation, is a crucial step in the crystallization process. When stone material supersaturates urine, it means that the concentration of ions in the urine exceeds their thermodynamic solubility as a result of the crystal formation. Large crystal nuclei cannot form unless they cling to the renal tubules and penetrate the renal pelvis. Within a few minutes, large crystals group together to form enormous clumps, which then change into larger crystals that eventually become retained in the urinary system [26]. Retention of crystals in the kidney causes damage to the tubular cells, which creates an environment that is favorable for the formation of new calcium oxalate nuclei on the surface of the kidney papillaries and facilitates the nucleation of crystals at lower supersaturation levels [27]. Treatments for urolithiasis that involve agents that block crystallization, modify these processes, or lessen oxalate supersaturation are very desirable. A variety of inhibitors can influence the nucleation, development, or aggregation of crystals [28]. The organic substances attach to the crystal's surface and prevent crystal nucleation, growth, and aggregation [29]. Numerous calcium oxalate inhibitors, including as glycosaminoglycans, magnesium, and citrate, are found in urine; however, due to their high molecular weight and restricted clinical application, these macromolecules are not elevated in urine. Certain treatments, such as sodium dodecyl sulphate [30, 31], metallic ions and their complexes [32], maleic acid copolymers [33, 34], and a human kidney protein [35], have been shown to have anti-crystallization properties in vitro; nevertheless, their practical applications are likewise restricted. According to conventional medicine, anti-lithogenic qualities can be demonstrated by either raising the volume and pH of the urine, which has an impact on the nucleation, development, and aggregation of crystals, or by balancing the processes that promote and inhibit crystallization in urine. Herbal medications provide a variety of phytoconstituents that help reduce the binding mucus of calculi or balance the crystalloid-colloid imbalance [36]. Studies in the literature suggest that the development of calcium oxalate monohydrate (COM) and calcium oxalate dehydrates (COD) crystals is significantly influenced by the pH of the urine. When the pH is between 5.5 and 7.0, more COM crystals form; when the pH is higher than 7.0, COD crystals form, but their number and width are smaller [37]. Due to M. uniflorum's historical use in kidney stones, the in vitro inhibitory impact of its aqueous extract on calcium oxalate crystallization was investigated in this work.

Urinary supersaturation and crystal formation are correlated, as was previously discussed. The nucleation formation rate increases in urine with increasing oxalate levels, so reducing supersaturation or oxalate levels is more successful in avoiding crystallization or nucleation. In the current investigation, as the concentration of sodium oxalate increases, seed extract inhibits the nucleation formation in a dose-dependent manner. The capacity of AEMU to form complexes with calcium and oxalate ions in solution and lower the supersaturation level may be the cause of this activity. But in both healthy and urolithiasis patients, nucleation formation is a common process. In healthy patients, small nuclei are typically easily excreted in the urine; however, in urolithiasis patients, after nucleation, free calcium and oxalate particles present in the urine attach to the preformed calcium oxalate crystal, increasing the size of the crystal. Calcium and oxalate concentrations affected the formation of crystals. The current investigation shown that AEMU inhibits crystal development in a dose-dependent way, although at the same extract dose, the inhibition rate reduced as the concentration of sodium oxalate gradually increased. Which indicated that extract may be prevent the growth by preventive the attachment of the small nuclei and remain separate from in the urine. Small crystals are readily excreted in urine, while large crystals are typically retained in renal tubules and contribute to the formation of stones when several crystals join together and bind to one another. As a result, it is believed that a crucial stage in the development of renal stones is the crystal aggregation process. In vitro calcium oxalate aggregation was reduced by AEMU in a concentration-dependent manner and reduced the crystal aggregation in renal epithelium which protect the renal damage and reduced the oxidative stress and pain due to stone. According to certain research, chemical treatments could be helpful in increasing the effectiveness of stone treatment by dissolving bigger, tougher stones. According to Zhou et al., buffered EDTA solvents could be a practical chemical treatment option for enhancing calcium oxalate stone dissolving efficacy [38]. The results of the current investigation showed that AEMU was effective in dissolving calcium oxalate stones in a concentration-dependent manner, suggesting that AEMU contains some of the ingredients needed to promote the breakdown of calcium oxalate stones and due to this effect during in vivo study small particles will be easily eliminate through the urine. AEMU's qualitative phytochemical examination showed the presence of phenolic chemicals, flavonoids, and saponins. These phytoconstituents are crucial in preventing the formation of urinary stones. Stone matrix's essential components, mucoproteins, are known to be broken down by saponins, which also have antilithic qualities [39]. Quercetin, rutin, diosmin, and hyperoside are known as flavonoids with high antioxidant and antilithiatic activities [40]. According to our analysis, AEMU has 36.44 ± 0.78 mg of diosgenin equivalent/g saponin and 4.72 ± 0.12 mg of quercetin equivalents/g flavonoids. Thus, the presence of such components in the AEMU may be the cause of the prevention of

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nucleation, crystal development, crystal aggregation, and dissolution. The current study solely examined the in vitro activity of AEMU; pre-clinical and clinical studies have not examined the drug's pharmacokinetics or pharmacodynamics. In order to confirm this, a well-known biomarker must be needed to distinguish herbal drugs from one another. To determine the pharmacokinetics and pharmcodynamic information of the active biomarker from the herb and its particular mechanistic pathway to avoid urolithasis, more in vivo tests and clinical investigations are required.

Conclusion

The in vitro initiation phases, such as nucleation and crystal aggregation, were found to be inhibited by the aqueous extract of M. uniflorum in a dose-dependent manner. It also demonstrated its effectiveness in inhibiting the growth and dissolution of crystals in a variety of ways. For these reasons, it can be confirmed that AEMU possesses antilithiatic properties, which may be brought about by the flavonoids and saponins that are found in AEMU. Our in vitro results clearly imply that AEMU should be evaluated for in vivo investigations in order to determine its precise activity and likely mode of action.

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Ethics Approval

Not Required.

Author contribution

Vaibhav Patel was performed the all-assay procedure, result analysis, Write up of manuscript. Niyati Acharya was finalized the all procedure, verify the result analysis, proof reading of manuscript.

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