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Original Research Article

In-vivo evaluation of anti-urolithiatic activity of methanolic extract of *Physalis minima* L. leaves against ethylene glycol induced urolithiasis in Wistar Albino rats

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ABSTRACT

Background: Urolithiasis, the formation of kidney stones due to crystallized urinary minerals, poses significant therapeutic challenges with conventional treatments often limited by adverse effects. Natural alternatives are increasingly sought, and *Physalis minima* L., known for its diuretic and anti-inflammatory properties, has shown promising phytochemical profiles that may counteract stone formation.
Methods: Male Wistar albino rats (n=30) were divided into five groups: a normal control, a lithiatic control (administered 0.75% v/v ethylene glycol with 1% w/v ammonium chloride for initial lithiasis acceleration), a standard group treated with Cystone (750 mg/kg), and two test groups receiving the methanolic extract of *Physalis minima* L. leaves (MEPM) at doses of 100 mg/kg and 200 mg/kg from days 15 to 28. Urine samples were analyzed for calcium, phosphate, and uric acid, while serum levels of blood urea nitrogen (BUN), creatinine, and uric acid were measured. Kidney tissues were subjected to histopathological examination to assess structural changes.
Results: Ethylene glycol administration significantly elevated urinary and serum stone-promoting markers. Treatment with MEPM, especially at 200 mg/kg, significantly reduced these biochemical parameters and improved kidney histology, displaying effects comparable to the standard Cystone treatment.
Conclusions: The methanolic extract of *Physalis minima* L. leaves exhibits potent, dose-dependent antiurolithiatic activity by lowering key stone promoters and restoring renal function. These findings highlight its potential as a natural therapeutic alternative for urolithiasis management, warranting further pharmacodynamic studies.

Keywords: Urolithiasis, *Physalis minima* L., Anti-urolithiatic, Kidney stones, Hypercalciuria

INTRODUCTION

Urolithiasis or nephrolithiasis, commonly known as kidney or renal stones which is refers to the multifaceted process of stone formation. Renal stones are composed of crystallized minerals that supersaturate the urine.[1] Development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, uric acid, cysteine, xanthine, and phosphate.[2] Urolithiasis occur in 1 of every 11 people in the United States at some time in their lifetimes, with men affected 2 to 1 over women. Kidney stone risk increases with age. The men who's older than 80 years have the highest incidence of urolithiasis. Unlike adults, in the paediatric age group, the highest incidence of nephrolithiasis is among female adolescents. Black and Hispanic populations have the lowest incidence of nephrolithiasis.[2]

The kidney stone formation process in humans are influenced by several factors known as promoters and inhibitors that either support or prevent the process of

stone formation thus affecting a person's ability to promote or prevent stone formation.[3] Stone promoters increase the likelihood of kidney stone formation. This includes uric acid, urine pH, urine volume hypercalciuria and hyperoxaluria.[3] Increased urinary super saturation (hyperoxaluria, hypercalciuria, low urine volume and increased urine pH) which initiate stone formation followed by crystal nucleation, crystal growth, crystal aggregation, crystal-cell interaction and crystal retention are the sequence involved in the stone formation.[4] Inhibitors are defined as molecules that increase the super saturation required to initiate nucleation, decrease crystal growth rate and aggregation, and inhibit secondary nucleation.[3] Stone inhibitors include alkaline pH, citrate, pyrophosphate, phytate, magnesium and glycoproteins.[3]

Treatment of renal stone depends on stone size and location. Many therapies like diuretics, citrate and chelating agent are given but they have their own pharmacological limitations, side effects on long use and not removing stones. So, in majority cases renal stone are removed by surgical treatment but that can cause side effects such as hypertension, tubular necrosis, haemorrhage and fibrosis of the kidney.[5] Efforts are made to find suitable curative agent for the treatment of urolithiasis from the natural resources like plants and animal origin. Synthetic drugs are highly complex, expensive and toxic. The search for natural product from plants possess potential therapy with less side effects compared to synthetic compounds and are strongly encouraged.

Recently human have more thirst towards natural medicines derived from the traditional knowledge of plant phytochemical and pharmacological properties. Herbal plants have a lots of medicinal activity like antioxidant, diuretic, antimicrobial, ant-inflammatory, hepatoprotective, antidiabetic, antiulcer, antipyretic, analgesic, antispasmodic properties, anti urolithiatic, etc. without side effects.

Plant phytochemical compounds such as flavonoids and phenolic agent have shown prophylactic and curative effects against kidney stones and have many health benefits.[1] Phytochemical present in plant play a crucial role in preventing and treating urolithiasis through diverse mechanisms. These mechanisms include antioxidant, anti-inflammatory and antimicrobial activities, along with the capability to hinder the formation and growth of crystals.[1] Aerva lanata, Berberis vulgaris, Hibiscus sabdariffa, Macrotyloma uniflorum, and Moringa oleifera are some traditional plants which have anti urolithiatic activity.[6-10]

Physalis minima L. belonging to the family solanaceae is an annual; small (1–meter height), delicate and erect plant.[11] *Physalis minima* L is an important medicinal plant in ayurvedic and Indian system of medicine. It is traditionally used as diuretic, smooth muscle relaxant, analgesic, anthelmintic, etc. *Physalis minima* L. plants contain secondary compounds, among other flavonoids, steroid alkaloids, phenolic compounds as ellagic acid, catechol, gallic acid etc.[12]

Based on the phytochemical compounds and invitro antiurolithiatic activity, *Physalis minima* L. leaves have been selected to investigate the existence of anti urolithiatic activity by invivo studies.[13]

METHODS

In the present study Albino Wistar rats served as an experimental animal.

Drugs & chemicals

Cystone – Apollo Pharmacy, ketamine HCL, methanol 99.95% and ethylene glycol were obtained from Naresh Scientific Company, Puducherry.

Study type

The study was an in vivo study and sub-acute model.

Study period

The study was conducted from September 2024 to October 2024.

Study place

The study was conducted in the animal house of Adiparasakthi College of Pharmacy, Melmaruvathur.

Collection, identification, and preparation of plant

The leaves of *Physalis minima* L. were collected from the cultivable lands, roadsides, river banks present around Vandavasi, authenticated by Dr. V. Gangadevi, M.Sc., Ph.D., Arignar Anna Government Arts College, Cheyyar. The Register number of the certificate: AAGAC/BOT/6/2024. The fresh leaves were collected in between April 2024 and June 2024. The collected leaves were further cleaned and shade dried. The dried material was coarsely powdered by means of mechanical grinder and passed through sieve No.10 and the powered material was used for the extraction process. Air dried coarsely powdered plant material (500 g) was defatted with petroleum ether. Powder of 100 g was packed in a soxhlet apparatus using methanol. The obtained methanolic extract of *Physalis minima* L. leaves was evaporated and concentrated. Based on the yield value, percentage yield was calculated, further the concentrated extract was subjected to preliminary phytochemical and pharmacological studies.

Phytochemical screening

The methanolic extract of *Physalis minima* L. leaves (MEPM) was subjected to preliminary phytochemical screening for the qualitative detection of phytoconstituents such as carbohydrates, alkaloids, glycosides, saponin, steroids, triterpenoids, phenolic compounds, tannins and flavonoids.

Experimental animals

Male Wistar Albino rats weighing 180-200 gm were procured from the "Mass Biotech", Chengalpattu, Tamil Nadu. Animals were placed in cages at room temperature (25±2ºC), relative humidity (55±5º) and 12 hr light-dark cycle with access to food pellet and water ad libitum. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC). (APCP/IAEC/2024-2025/8, dated 13th may 2024).

Ethylene glycol induced urolithiasis in rat

A 30 male Wistar Albino rats were divided into 5 groups containing 6 animals in each group.

Group I served as a normal control and received normal saline 0.9% v/v p.o.

All remaining groups received calculi inducing treatment for 28 days, comprised of 0.75% v/v ethylene glycol with 1% w/v ammonium chloride using drinking water for first 3 days to accelerate lithiasis followed by only 0.75% v/v ethylene glycol for 28 days.

Group II served as lithiatic control

Group III served as standard and received the standard drug "Cystone" at dose of 750 mg/kg from 15th day to 28th days.

Group IV served as test group (Low dose) and received MEPM at doses of 100 mg/kg from 15th day to 28th day.

Group V served as Test group (High dose) and received MEPM at dose of 200 mg/kg from 15th day to 28th day.14,5

The methanolic extract of Physalis minima L. leaves 100mg/kg and 200mg/kg was dissolved in distilled water and was given once p.o for 14 days.

Urine collection and analysis

Animals were kept in the metabolic cages (3 animals/metabolic cage) on the 28th day and 24 hrs urine samples were collected. Animals had free access to drinking water during the urine collection period. Urine was analyzed for calcium, phosphate and uric acid. The urine sample was kept at -20 °C until analyzed.

Serum collection and analysis

At the end of the treatment period, under anaesthetic condition (Ketamine HCL 22 mg/kg -I.M) blood was collected from the animal by cardiac puncture, serum was separated by centrifugation at 10,000 g for 15 minutes and analyzed for blood urea nitrogen (BUN), creatinine and uric acid.

Histopathological analysis

To confirm the incidence of urolithiasis the animals were euthanized by ketamine overdose and kidneys were isolated, washed, weighed and kept quickly in 10 % formalin and subjected to histopathological studies using Hematoxylin and Eosin stains (H&E).

Statistical analysis

The data obtained were expressed as mean ± SEM and analyzed using one-way ANOVA followed by Dunnet 't' test using Graph pad prism software (version 10).

RESULTS

Phytochemical screening

The MEPM was qualitatively analyzed for various phytoconstituents and the results revealed presence of alkaloids, steroids, tannins, flavonoids, phenolic compounds and the absence of carbohydrates, glycosides, saponin.

Biochemical analysis

Effect on urine parameters

The level of urine calcium, phosphate and uric acid in Group I to V is shown in Table 1. In ethylene glycol treated group (Group II), the level of calcium, phosphate and uric acid showed significant increase ($p<0.001$, $p<0.0001$ and $p<0.001$) respectively when compared to normal control (Group I). However, treatment with MEPM (100 and 200 mg/kg) and Cystone (750 mg/kg) significantly reduced the levels of calcium, phosphate and uric acid when compared to the lithiatic control group (Group II) (Figure 2).

Effect of serum parameters

The level of BUN, creatinine and uric acid in Group I to V is shown in Table 2. In ethylene glycol treated group (Group II), the level of BUN, creatinine and uric acid showed significant increase ($p<0.001$, $p<0.0001$ and $p<0.001$) respectively when compared to normal control (Group I). However, Treatment with MEPM (100 and 200 mg/kg) and Cystone (750 mg/kg) significantly reduced the levels of BUN, Creatinine and uric acid when compared to the lithiatic control group (Group II) (Figure 2).

Histopathology of kidney

Microscopical observation of normal control group shows intact tubular epithelium and glomerular structures (Figure 1(a)). Lithiatic control shows multifocal areas revealed presence of crystals within the tubules with moderate to severe degeneration of tubular epithelium, Multifocal congestion of blood vessels, intertubular haemorrhages and glomerular blood vessel with congestion, mild dilatation of tubules observed (Figure 1(b)). Standard group shows mild congestion of blood vessels, mild degeneration of tubular

epithelial cells, distension and dilatation of tubules with interstitial infiltration of mononuclear cells and haemorrhages (Figure 1(c)). MEPM (100 mg/kg) treated group shows multifocal areas revealed moderate interstitial mononuclear cell infiltration with degeneration and necrosis of the tubules along with haemorrhages. Few areas revealed eosinophilic exudate (oedema) (Figure 1(d)). MEPM (200 mg/kg) treated group multifocal areas revealed mild distention of tubules, very mild infiltration of mononuclear cells, mild intertubular haemorrhages (Figure 1(e)).

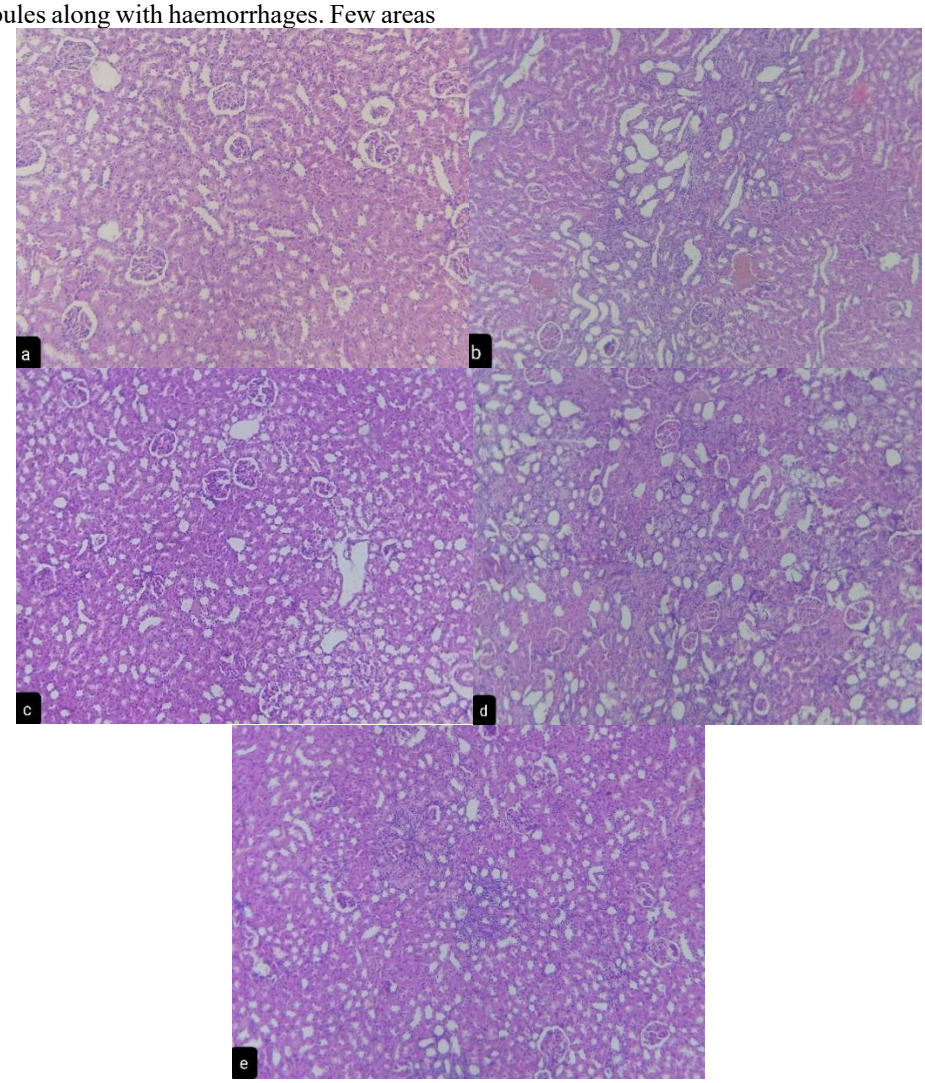

Figure 1: Histology of kidney (a) Normal control group (X10 H&E), (b) Lithiatic control group (X10 H&E), (c) Standard group (cystone – 750 mg/kg) (X10 H&E), (d) MEPM (100 mg/kg) treated group (X10 H&E), (e) MEPM (200 mg/kg) treated group (X10 H&E).

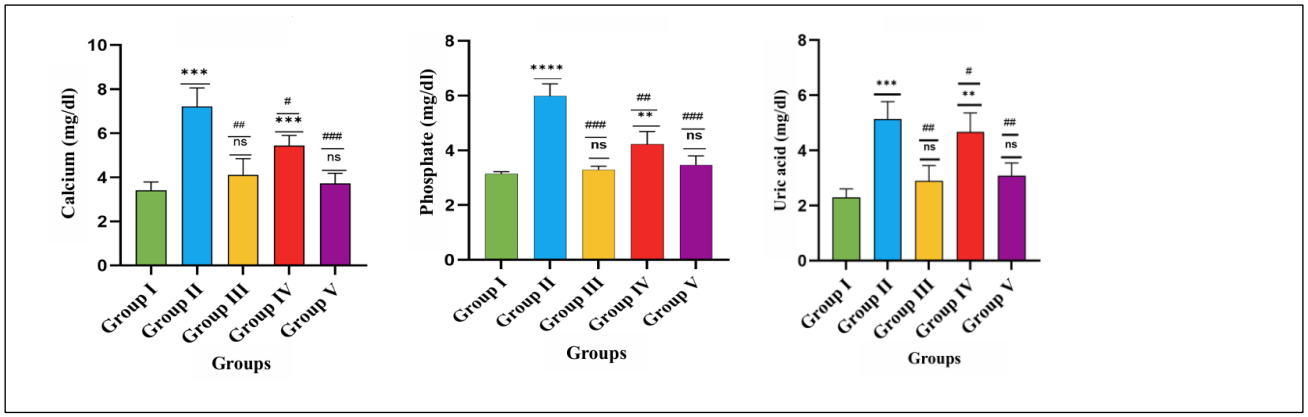

Figure 2: Effect of MEPM in urine parameters.

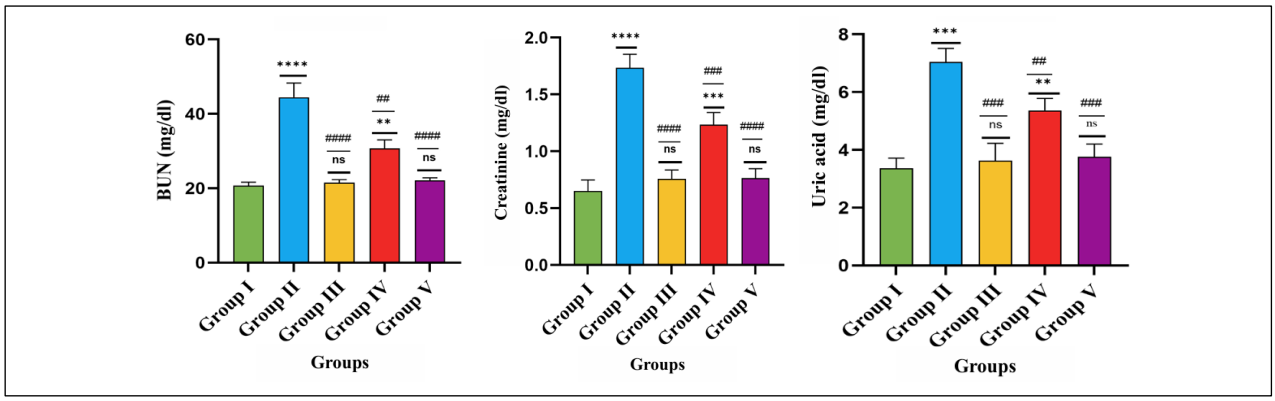

Figure 3: Effect of MEPM in serum parameters.

Table 1: Effect of MEPM on urinary parameters in urolithiatic male Wistar rats.

Groups	Calcium (mg/dl)	Phosphate (mg/dl)	Uric acid (mg/dl)
Group I - Normal Control	3.41±0.37	3.15±0.07	2.29±0.30
Group II - Lithiatic control	7.20±0.84***	5.99±0.44****	5.14±0.63***
Group III- Standard group (cystone – 750 mg/kg)	4.12±0.72^{ns} 4.12±0.72$^{\#\#}$	3.30±0.13^{ns} 3.30±0.13$^{\#\#\#}$	2.89±0.55^{ns} 2.89±0.55$^{\#\#}$
Group IV- MEPM 100 mg/kg	5.44±0.46*** 5.44±0.46$^{\#}$	4.22±0.46** 4.22±0.46$^{\#\#}$	4.67±0.69** 4.67±0.69$^{\#}$
Group V - MEPM 200 mg/kg	3.71±0.47^{ns} 3.71±0.47$^{\#\#\#}$	3.46±0.33^{ns} 3.46±0.33$^{\#\#\#}$	3.09±0.45^{ns} 3.09±0.45$^{\#\#}$

All values are mean ± SEM (n = 6), one-way ANOVA followed by Dunnet 't' test; * - Comparisons are made with Normal control; # - Comparisons are made with Lithiatic control; */# = p<0.05, **/# # = p<0.01, ***/### = p<0.001, ****/#### = P<0.0001, ns = Non significant.

Table 2: Effect of MEPM on serum parameters in urolithiatic male Wistar rats.

Groups	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group I - Normal control	20.74±0.88	0.65±0.09	3.36±0.35
Group II - Lithiatic control	44.48±0.81***	1.73±0.12****	7.04±0.48***
Group III- Standard group (cystone - 750mg/kg)	21.46±0.84^{ns} 21.46±0.84$^{\#\#\#}$	0.76±0.08^{ns} 0.76±0.08$^{\#\#\#\#}$	3.63±0.60^{ns} 3.63±0.60$^{\#\#\#}$
Group IV-MEPM 100 mg/kg	30.74±2.26** 30.74±2.26$^{\#\#}$	1.23±0.11*** 1.23±0.11$^{\#\#\#}$	5.37±0.41** 5.37±0.41$^{\#\#}$
Group V -MEPM 200 mg/kg	22.14±0.70^{ns} 22.14±0.70$^{\#\#\#}$	0.76±0.08^{ns} 0.76±0.08$^{\#\#\#\#}$	3.76±0.44^{ns} 3.76±0.44$^{\#\#\#}$

All values are mean ± SEM (n = 6), one-way ANOVA followed by Dunnet 't' test; *- Comparisons are made with Normal control; # - Comparisons are made with Lithiatic control; */# = p<0.05, **/# # = p<0.01, ***/### = p<0.001, ****/#### = p<0.0001, ns = Non significant.

DISCUSSION

Stones formed in the kidneys of rats and humans are identical at the ultra-structural level in nature and composition of their matrix, thus rat models of urolithiasis are helpful experimental tools for exploring the pathophysiology of the disease.[5]

In the present experiment, male Wistar rats were chosen to induce urolithiasis with 0.75%v/v ethylene glycol with 1% w/v ammonium chloride for 3 days to accelerate lithiasis and followed by only 0.75% v/v ethylene glycol for 28 days. The urinary system of male rats resembles that of humans, and previous studies have proved that stone formation in female rats was considerably less as compared to the male rats.

It has been reported earlier that ethylene glycol causes hypercalciuria, hyperphosphaturia and hyperuricemia leading to urolithiasis. 10 After 28 days in the current investigation, we also found that a significant rise in urine calcium, phosphate, and uric acid was observed in the disease control rats, showing that supersaturation of lithogenic ethylene glycol, which may result in the increased calcium occurs due to excessive tubular damage in the kidney, leading to excretion of intracellular calcium

via urine as compared to normal group, maybe the source of the renal damage.[19]

As associated with the normal control group, treatment with MEPM 200 mg/kg decreases the incidence of stone development and significant decreases calcium, phosphate and uric acid excretion in urine by $p<0.001$, $p<0.001$ and $p<0.01$. These results were in agreement with other previous reports.

Due to the obstruction caused by urinary system stones in urolithiasis, GFR is diminished, and as a result, nitrogenous waste products including creatinine, BUN and uric acid build up in the blood. 20 In the current investigation, the urolithiatic effects of ethylene glycol were characterized by significant increases in serum creatinine, BUN and uric acid.

As associated with the normal control group, treatment with MEPM 200 mg/kg showed significant decreases creatinine, BUN and uric acid levels in serum by $p<0.0001$, $p<0.001$ and $p<0.001$. These results were in agreement with other previous reports.

And when associated with the normal control and diseased control, treatment groups showed an enhanced glomerular filtration rate and, thus, decreased the deposition of nitrogenous waste products serum BUN, creatinine and uric acid.

The biochemical analyses were also aided by the histopathological examinations of the kidney. Microscopic examinations of the kidney sections of the stone-induced groups showed crystal deposit, moderate to severe degeneration of tubular epithelium, congestion and dilation of blood vessels. As associated with the normal control group, treatment with the MEPM 200 mg/kg and cystone was showed mild distention of tubules, very mild infiltration of mononuclear cells, mild intertubular haemorrhages.

CONCLUSION

On the basis of above result and discussion it can be concluded that, the methanolic extract of *Physalis minima* L. leaves (MEPM) possess potent antiurothiatic activity against ethylene glycol induced urolithiasis in albino rats in a dose dependent manner. The antiurolithiatic effects may be mediated possibly through decreasing the concentration of various stone promoters like calcium, phosphate, uric acid in urine and BUN, creatinine, uric acid in serum. The therapeutic effect of leaf extract may also be due to diuretic property or dissolution of kidney stones formed or may be synergistic effect of all. Thus, the findings of the present study emphasize the MEPM possess potential medicinal value and is beneficial in treatment of urolithiasis. Further investigation on pharmacodynamic parameter of *Physalis minima* L. to be carried out in future to find appropriate mechanism responsible for the antiurolithiatic activity.

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