



***IN-VITRO* ANTIUROLITHIATIC ACTIVITY OF SUCCESSIVE
LEAF EXTRACTS OF *Physalis minima* Linn**

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Received 25th May 2023; Revised 26th July 2023; Accepted 1st Sept. 2023; Available online 1st May 2024

<https://doi.org/10.31032/IJBPAS/2024/13.5.8043>

ABSTRACT

Urolithiasis is a process of stone formation in the urinary tract, affecting the kidneys, ureters, bladder or urethra. It is also termed as nephrolithiasis, urolithiasis or ureterolithiasis with a male- female ratio of 2:1. In the present study, we evaluated the *in-vitro* anti-urolithiatic activity of *Physalis minima* Linn. The shade dried and powdered plant material was subjected to cold maceration of coarsely powdered leaves of *Physalis minima* Linn., using n-hexane, ethyl acetate and ethanol. The extracts were then subjected to preliminary phytochemical screening, followed by *in-vitro* antioxidant and antiurolithiatic activity. The qualitative phytochemical results revealed the presence of flavonoids, phenol, sugar, lipids, steroids, tannins, saponins, fat, alkaloids and lignin. *In-vitro* antioxidant activity, determined by hydrogen peroxide assay, showed the ethanol extract with least IC₅₀ value of 51.02µg/mL. Further the *in-vitro* anti-urolithiatic activity revealed that the ethanol extract of *Physalis minima* Linn., showed better antiurolithiatic activity than n-hexane and ethyl acetate extracts. Thus current study validates the antiurolithiatic potential of *Physalis minima* Linn., in the inhibition of stone formation and dissolution of stone crystals which might be due to the antioxidant property.

Keywords: *Physalis minima*, hydrogen peroxide assay, urolithiasis

INTRODUCTION

Urinary calculi are the urinary system's third most frequent disorder. Calcium oxalate generates nearly 80% of these calculi [1]. The etiology of urinary stone formation is multifactorial. It affects both men and women, while men are at a higher risk [2]. Calcium oxalate monohydrate, apatite, calcium hydrogen phosphate, and calcium carbonate are the components of calcium calculi [3]. Citrate and magnesium are the two main inhibitors of stone formation in the urinary tract, and low levels or a lack of these inhibitors in the urine leads to stone formation [4]. Several pharmacological and clinical studies on traditional medicinal plants used in the treatment of kidney stones have demonstrated that their therapeutic potential in both *in-vitro* and *in-vivo* models. Various herbal plants such as *Pergularia daemia* [5], *Abelmoschus moschatus* [6], *Mimusops elengi* [7], have successfully proved as prophylactic and curatives for urolithiasis.

Physalis minima Linn (Solanaceae) is an annual herbaceous plant, found throughout tropical and subtropical regions of the world [8]. *Physalis minima* L. is widely distributed in the southeast and southwest of China and is used to treat a variety of illnesses, including urinary diseases, spleen ailments, cancer, and inflammation [9]. The plant is reported with several metabolites including phenolics,

alkaloids, steroids, and flavonoids [10]. The predominant phytoconstituents include, 5-Methoxy-6,7-methylenedioxyflavone, 5,6,7-trimethoxyflavone, withaphysalin A and withaphysalin D [10-12]. Recent scientific investigation has also demonstrated anti-inflammatory [13], analgesic [14], antipyretic [14] and anticancer [15] activities.

In a study by Sudhakaran *et al.*, oral administration of petroleum extract of *P. minima* L. exhibited antifertility effect in female albino rats with altered histoarchitecture of the ovary, oviduct and uterus. Particularly, the histopathological alterations like absence of corpora lutea and further follicular irregularities which might be attributed to the inhibition of gonodotrophins, and direct inhibitory effects on female reproductive system [16]. In another study by Sowmiya et al., 2021, the crude methanolic leaf extract upon fractionation with n-hexane, chloroform and ethanol revealed a total of 35, 40, and 38 components with a total peak area of 97.35%, 97.24%, 98.16% respectively [17].

Furthermore, a study by Joseph and Ravi, 2022 has recorded the neuroprotective effects of fruits of *P. minima* L. on rats in D-galactose induced Alzheimer's model. Moreover, the study revealed the enriched presence of 0.96 and 0.974 milli equivalence of flavonoids and phenolics justifying their

promising role as an antioxidant, acetylcholinesterase inhibitor and in the treatment of Alzheimer' s disease [18]. Additionally, the *in-vitro* antioxidant activity studied by Swetha et al., has indicated that the methanolic extract of *P. minima* L. leaf showed higher potential compared to other extracts of stem and leaves [19]. With a view to explore the traditional usage as antiurolithiatic, the present study was directed at evaluating the *in-vitro* anti-urolithiatic activity of leaves of *P. minima* along with its hydrogen peroxide radical scavenging effect.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

All the chemicals and reagents used in the study were procured from certified suppliers and were of the highest analytical grade. The polyherbal drug cystone was purchased from the Himalayas drug company, Bengaluru.

COLLECTION OF PLANT MATERIAL AND ITS AUTHENTICATION

Leaves of *Physalis minima* Linn., (Solanaceae) were collected from Thirukkalukundram, Tamil Nadu during March 2021. Fully developed leaves were collected in a fine, dry climate and stored in a shed for three weeks in a loose stack. The plant was identified and authenticated by Plant Anatomy Research Centre, Chennai, No. PARC/2021/4458. The leaves were coarsely powdered and used for further

research after being shade dried. A voucher specimen has been reserved in the Department of Pharmacognosy, SRM College of Pharmacy, Chennai.

PREPARATION OF EXTRACT

Extraction is the preliminary step involved in the phytochemical studies. It brings out the metabolites into the extracting solvent. Solubility of the metabolite depends upon its polarity. Extraction was performed by cold maceration of coarsely powdered leaves of *Physalis minima* Linn., with solvents off n-hexane, ethyl acetate and ethanol [20]. The extract was then concentrated by distilling of the solvent and evaporated to dryness and the percentage yield was calculated. The shade dried coarsely powdered leaves of *Physalis minima* Linn., was subjected to the further analysis.

PRELIMINARY PHYTO CHEMICAL ANALYSIS

Preliminary phytochemical screening was carried out to identify the presence of various phytochemical constituents in all three extracts [21]. The n-hexane, ethyl acetate and ethanol extracts of *P. minima* L. leaves were subjected to phytochemical analysis using different chemical tests provided in the standard protocols for the presence of alkaloids, carbohydrates, glycosides, cardiac glycosides, steroids, proteins, flavonoids, and quinones.

QUANTITATIVE ESTIMATION OF PHENOLIC CONTENT

About 100 μ L of extracts (1 mg/mL) were treated with 0.5 mL of Folin Ciocalteu reagent, 400 μ L of 20% sodium carbonate, 5 mL of distilled water and kept at room temperature for 30 minutes. Gallic acid was used as standard and absorbance read at 760nm [22].

HYDROGEN PEROXIDE SCAVENGING ASSAY

Various concentrations of the extracts were added to 0.6 mL of 43 mM hydrogen peroxide in 1M phosphate buffer solution (pH 7.4). Ascorbic acid was used as standard and the absorbance was read at 230 nm after 10 minutes with phosphate buffer as blank [23]. The hydrogen peroxide scavenging activity was calculated using the formula,

$$\text{Percentage inhibition} = [(\text{Control} - \text{Test}) / \text{Control}] \times 100.$$

IN-VITRO ANTIUROLITHIATIC ACTIVITY

Calcium oxalate method

One mL of calcium chloride dehydrate (0.025 M) was mixed with 2 mL of Tris-buffer (pH 7.4) and 1 mL of various concentrations of ethanol extract of *Physalis minima* (500, 250, 100, 50, and 10 μ g/mL). About 1 mL of 0.025 M sodium oxalate was added to each test tube and the mixture was then incubated at 37 °C for 24 hours. Absorbance was read at 620 nm and cystone was used as standard [24]. The inhibition in

the formation of stone nucleus was calculated by using the formula,

Percentage of inhibition

$$= \frac{\text{Absorbance control} - \text{Absorbance test}}{\text{Absorbance control}} \times 100$$

RESULTS

PRELIMINARY PHYTOCHEMICAL ANALYSIS

Qualitative preliminary phytochemical analysis was carried out in all the three extracts. The n-hexane extract showed the presence of steroids. The ethyl acetate extract showed the presence of alkaloids, tannins, proteins and flavonoids. The ethanol extract showed the presence of carbohydrates, proteins, tannins and flavonoids.

QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC CONTENT

The total phenolic content was determined by UV spectrophotometry. The total phenolic content of the ethyl acetate and ethanol extracts of *Physalis minima* were given in (Table 1). The total phenolic content was determined by standard (gallic acid) calibration curve with the regression equation of $y = 0.861 * x + 0.831$ and a correlation coefficient (r^2) 0.931. The ethanolic extract showed the highest phenolic content of 0.65mg of gallic acid equivalents / g dry extract in comparison to the ethyl acetate extract that showed the phenolic content of 0.43mg of gallic acid equivalent /g dry extract.

HYDROGEN PEROXIDE SCAVENGING EFFECT OF *Physalis minima*

The hydrogen peroxide scavenging activity of the different extracts of *Physalis minima* at different concentrations were tabulated in **Figure 1**. The hydrogen peroxide scavenging activity with decreasing IC₅₀ values were as follows – ethanol extract (51.02 µg/mL) > n-hexane extract (66.71 µg/mL) > ethyl acetate extract (128.4 µg/mL). The results revealed that the ethanol extract showed the highest scavenging ability and was comparable with the ascorbic acid which possessed the least IC₅₀ value of 42.42 µg/mL.

ANTIUROLITHIATIC EFFECT OF ETHANOL EXTRACT OF *Physalis minima*

The percentage inhibition exhibited by the ethanol extract of leaves of *Physalis minima* in the formation of stone nucleus as determined by calcium oxalate method. Based on the results obtained from hydrogen peroxide scavenging activity of various extracts of *Physalis minima*, the ethanol extract was selected for the further antiurolithiatic activity *in-vitro*. The results showed that the ethanol extract of leaves of *P. minima* produced a significant percentage inhibition of 66.68 % at the highest concentration of 500 µg/mL as compared to the standard cystone (92.09%) as depicted in **Figure 2**.

Table 1: Quantitative estimation of phenolic content of leaf extracts of *Physalis minima*

S.No.	Extracts of <i>Physalis minima</i>	Total phenolic content (milligrams of GAE/g of dry extract)
1.	Ethyl acetate extract	0.43 ± 0.08
2.	Ethanol extract	0.65 ± 0.09

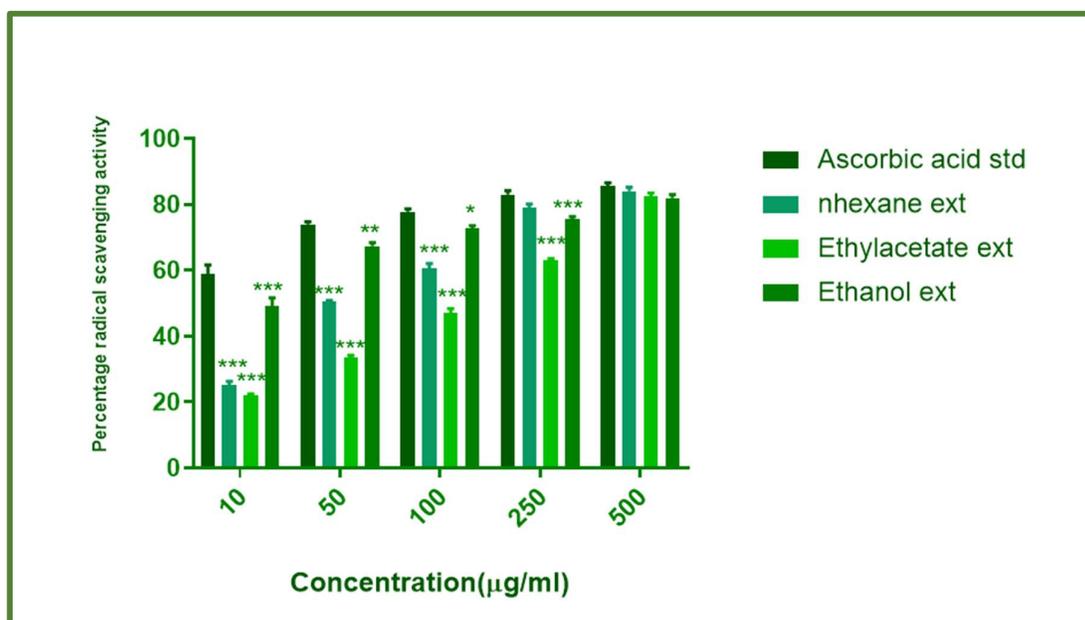


Figure 1: Hydrogen peroxide scavenging activity of the different extracts of *Physalis minima*. Values were expressed as Mean±SEM; n=3, Two way ANOVA followed by Bonferroni's multiple comparison test. ***p<0.001, **p<0.01, and *p<0.1 compared to standard Ascorbic acid

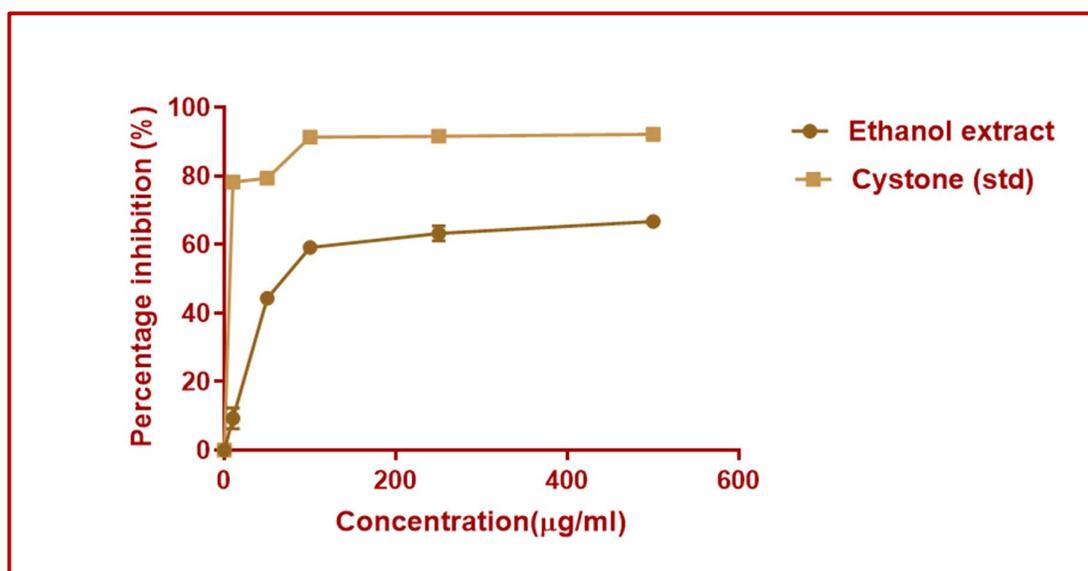


Figure 2. Percentage of inhibition of ethanol extract of *Physalis minima* on calcium oxalate crystal. Values were expressed as Mean±SEM; n=3, Two way ANOVA followed by Bonferroni's multiple comparison test. ***p<0.001, **p<0.01, and *p<0.1 compared to standard cystone

DISCUSSION:

Urinary stones are the hard and crystalline minerals resulting from an imbalance between the inhibitors and promoters of urinary calculi. Moreover, urinary supersaturation can also be considered as a major factor for the formation of calculi [25]. In addition, Reactive oxygen species play a vital role in the crystallization and aggregation of calcium oxalate crystals. The renal epithelial cells generate ROS on exposure to calcium oxalate crystals. Continuous exposure to Calcium oxalate crystals causes injury to the renal cells that are further injured by oxidative stress thereby enhancing lipid peroxidation and weakening the renal antioxidant defense system [26].

In the present study, we carried out the preliminary phytochemical screening,

quantitative phenolic content and hydrogen peroxide scavenging effect of various extracts of leaves of *P. minima*. Based on the *in-vitro* antioxidant results carried out by hydrogen peroxide assay, the ethanol extract was selected for the further *in-vitro* antiurolithiatic activity by turbidimetry method and the results were compared to standard cystone. Cystone stands as a promising herbal formulation consisting of *Didymocarpus pedicellata*, *Saxifraga ligulata* and Gokshura used in urinary tract infections and urolithiasis [27].

Our results indicated the presence of varied phytoconstituents in the different extracts of leaves of *P. minima* upon preliminary phytochemical test. Further, the extracts showed an appreciable phenolic content providing a better clue on the phytoconstituents possessing free radical

scavenging ability [28]. Furthermore, out of the three extracts, the ethanol extract showed the highest hydrogen peroxide radical scavenging effect. The anti-urolithiatic activity carried out on the ethanol extract of *P. minima* showed a promising effect with an IC₅₀ value of 67.3 µg/mL in comparison with standard (IC₅₀ value of 7.33 µg/mL). Several reports reveal the varied biological activities of various extracts of *P. minima* including antioxidant, antibacterial, anti-inflammatory, analgesic, antidiabetic and antidiuretic activity [29]. The present study records the hydrogen peroxide scavenging activity and anti-urolithiatic activity of *P. minima* and further investigations are directed towards *in-vivo* studies.

CONCLUSION

The study was intended with a view to evaluate the *in-vitro* antioxidant activity was determined by H₂O₂ radical scavenging assay and anti-urolithiatic activity by turbidity method. Cold maceration with solvents of increasing polarity resulted in n-hexane, ethyl acetate and ethanol extracts. Among the three extracts, the ethanol extract showed stronger antioxidant potential. Further when subjected to turbidity assay, the ethanolic extract of *Physalis minima* Linn., showed a promising antiurolithiatic activity and further studies are directed for the development into a suitable formulation.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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