



Antiurolithiatic potential of selected ethnomedicinal plants: a comparative *in-vitro* study

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Abstract

Urolithiasis is a common and recurrent health problem for which traditional medicinal plants have been widely used. *Impatiens psittacina*, *Withania somnifera* and *Kalanchoe pinnata* are traditionally prescribed by folklore practitioners of Golaghat district, Assam, for treating urolithiasis. The present study evaluated their *in vitro* antiurolithiatic activity using aqueous and methanolic extracts. Calcium oxalate (CaOx) stones were prepared artificially, and Cystone was used as the positive control. Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, terpenoids, steroids, tannins and glycosides in varying combinations. Methanolic extracts showed higher dissolution activity than aqueous extracts. At 30 mg/ml of methanolic extract, *Withania somnifera* showed the highest dissolution potential (81.33%), followed by *Kalanchoe pinnata* (80.66%) and *Impatiens psittacina* (77.66%), compared with Cystone (82.33%). The findings suggest that these plants possess promising *in vitro* antiurolithiatic activity and may help validate traditional medicinal knowledge.

Keywords: Cystone, Folklore practitioners, *Impatiens psittacina*, *Kalanchoe pinnata*, Urolithiasis, *Withania somnifera*.

Introduction

Traditional herbal medicines, also known as ethnomedicine, folk medicine or indigenous medicine, comprise medical knowledge that developed over generations before the era of modern conventional medicines. It is the oldest form of treatment that is based on underlying philosophy and set of principles by which it is practiced. Traditional healing practice was formerly an integral part of semi-nomadic and agricultural indigenous societies and although archeological confirmation for its existence dates back to only around 6000 BC [1]. According to the World Health Organization (WHO), traditional medicine is a health practice that integrates plants, animals, and mineral-based medicines, spiritual therapies, manual techniques, and exercises, applied singularly or in combination to cure, diagnose, and prevent illness or maintain well-being [2]. Ayurveda, a 5000-year-old medical system in India, emphasizes the role of the body, mind, and spirit in disease prevention and treatment through diet and herbal remedies [3]. Nowadays these herbal therapies are gaining significance among public for curing various ailments including remedies for urolithiasis. Urolithiasis (also called renal calculi, nephrolithiasis or kidney stones), is the process of formation of liths or stones in the renal tract by crystallization of some chemical compounds found in the urine. It is the third most prevalent urinary disorder with recurrence rate of 50% [4]. Urolithiasis occurs as a result of nucleation, aggregation, supersaturation, growth and retention of uroliths within the renal tubules [5]. Renal stones are mainly composed of calcium oxalate (CaOx), uric acid, struvite, and cystine [6]. Calcium containing stones are the most common comprising about 75% of all urinary calculi which may be present in the form of pure calcium oxalates (50%) or calcium phosphate (5%) and a mixture of both (45%). Calcium oxalate stones are found in two forms: Calcium Oxalate Dihydrate (COD), Calcium Oxalate Monohydrate (COM), and weddellite [7].

Modern techniques such as Extracorporeal Shock Wave Lithotripsy (ESWL), Percutaneous Nephrolithotomy (PNL), ureteroscopy (URS), and open surgery are used if the renal stone size is more than 5 mm. However, these modern therapies have shown significant side effects, such as renal tissue necrosis, renal damage, hypertension, and damage to surrounding organs [8]. However, the constant exploration of new antiurolithiatic drugs from natural sources is of greater significance, as herbal drugs are cost-effective and may show fewer side effects in the future than the synthetic drugs available in the market.

There are many herbal plants that have the potential to dissolve or break the stones formed in the urinary tract such as *Impatiens psittacina* (Hook.f), *Withania somnifera* (L.) Dunal and *Kalanchoe pinnata* (Lam.) Pers. *I. psittacina*, also known as parrot balsam, belongs to the family Balsaminaceae. It is also known as the Thailand Parrot Flower because of its resemblance to a flying cockatoo. *W. somnifera*, belonging to the family Solanaceae, has long been used in Indian traditional medicine to cure many diseases, such as rheumatism, dyspepsia, and anthelmintic [9]. Ashwagandha is commonly known as “Indian Winter cherry” or “Indian Ginseng”. It is one of the most important herbs in Ayurveda, used for millennia as a Rasayana (tonic) for its wide-ranging health benefits [10]. *K. pinnata*, commonly known as Patharchatta, belongs to the Crassulaceae family and is a perennial herb that grows widely and is traditionally used in tropical Africa, America, Australia, India, and China. *K. pinnata* consists of various active compounds including alkaloids, triterpenoids, flavonoids, glycosides, steroids, lipids, bufadienolides and organic acids etc (Kumar *et al.*, 2023)

An ethnobotanical study in the Golaghat district of Assam revealed that folk medicine practitioners or herbal practitioners prescribe *I. psittacina*, *W. somnifera*, and *K. pinnata* for the treatment of urinary stones. Thus, the aim of the present study was to evaluate the potential of extracts from all three plants for their antiurolithiatic activity using *in vitro* methods.

Materials and methods

Study area

The Golaghat district (26.0°-27.1°N latitude and 93.0°-94.18° E longitude) in Assam, India, is situated at approximately 100 meters above sea level and has an area of approximately 3502 sq. km (Fig1). The climate of the entire district is monsoon, which facilitates the luxuriant growth of various types of plants, from lower cryptograms to higher angiosperms [11]. The present fieldwork was carried out among the traditional healers or herbal practitioners of Golaghat district for the local herbal plants used for the treatment of urolithiasis.

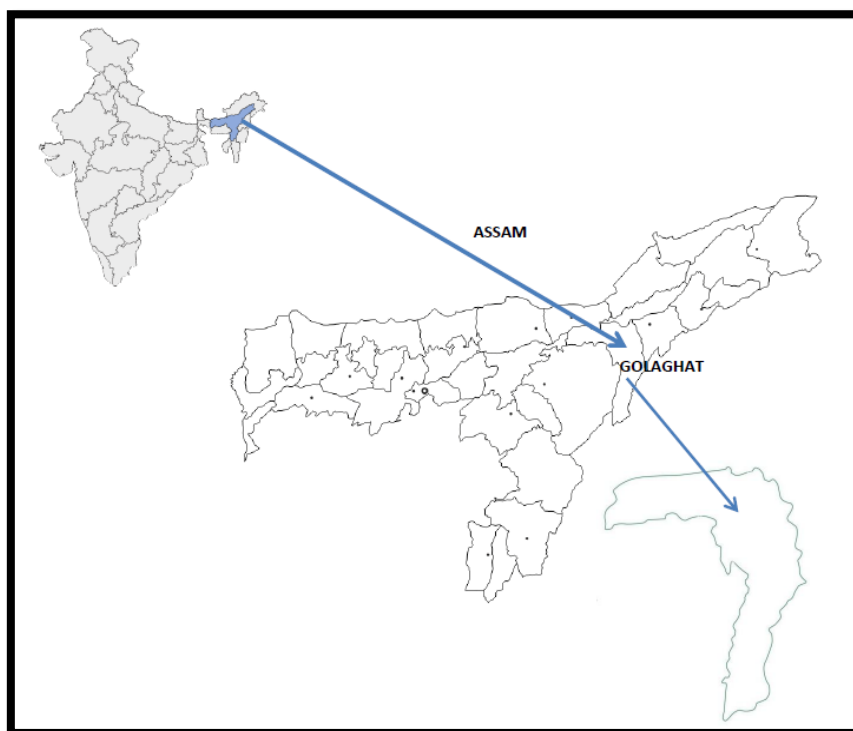


Fig.1. Map of Golaghat District, Assam, India.

Collection of data and selection of medicinal plants

Field work was conducted among traditional healers from November 2020 to July 2022. For the collection of information on its use, senior villagers who were associated with these healers were consulted through repeated interviews and questionnaires. Among other medicinal plants used for the treatment of various ailments, herbal practitioners in this region prescribe *Impatiens psittacina*, *Withania somnifera*, and *Kalanchoe pinnata* for the treatment of kidney stones. Consistent with the study objective, plants reported by traditional healers as effective against kidney stones were selected for evaluation, with no scientific evidence of antiurolithiatic efficacy. Other medicinal plants were not considered for further investigations for the obvious reasons that the plants are not prescribed for treatment of kidney stones.

Collection and preparation of plant extract

Plant materials were collected, along with photographic data, and relevant information disclosed by the traditional healers was recorded. The collected plant materials were shade-dried and ground into a fine powder using a mortar and pestle. The extracts were prepared using water and methanol solvents along with powdered plant material using a Vacuum Evaporator and Soxhlet Extraction method [12].

Phytochemical screening

The crude extracts were screened for the presence of phytochemicals by following standard protocols as described by Sofowora [13], Trease and Evans [14], [15].

Preparation of calcium oxalate crystals

Calcium oxalate crystals were prepared by taking equimolar solution of Calcium chloride dihydrate (A.R) which was dissolved in distilled water and Sodium oxalate (A.R) in 10 ml of 2N sulphuric acid (H_2SO_4) and both were allowed to react in a beaker (Fig 2.a). The resulting precipitate of calcium oxalate was washed with 10% ammonia solution to make the precipitate free from traces of H_2SO_4 . Then again it was washed with distilled water for about 4-8 times and was allowed to air dry for some time. After that it was kept in deep freezer for 24 hours and then allowed to dry at a temperature of 60 °C for 4 hours (Fig 2.b) [16].

The growth of crystal was expected due to the following reaction:

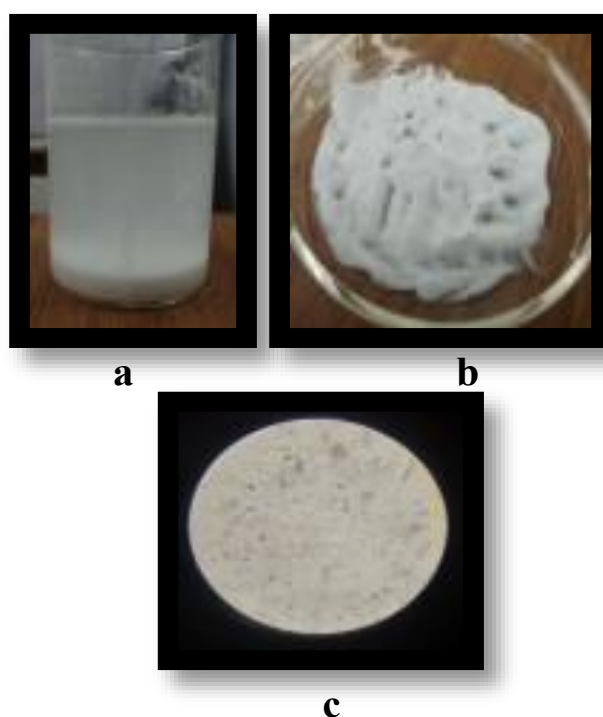
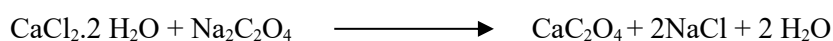


Fig. 2. Preparation of oxalate stones. a. Preparation of Calcium oxalate; b. Precipitates of Calcium oxalates; c. Calcium oxalate crystals (400x).

Preparation of the Semi permeable membrane from farm eggs

Outer calcified shell of eggs was chemically removed by placing the eggs in 2 M HCL for overnight, which caused complete decalcification (Fig 3). After that the membrane was prepared by the method as explained by Atodariya *et al.* [16].



Fig. 3. Decalsification of egg shell.

Estimation of Calcium oxalate by Titrimetric method

Exactly 1mg/ml of the calcium oxalate (CaOx) and different concentrations of extract i.e., 10 mg/ml, 20 mg/ml and 30 mg/ml were packed together in the semi permeable membrane. Standard drug Cystone (Himalaya) served as positive control (taken in a concentration of 10mg/ml) and one group as negative control (contained only 1mg of calcium oxalate in distilled water). This was then allowed to suspend in a conical flask containing 100 ml 0.1 M TRIS buffer as shown in Fig 4. The conical flasks were then incubated in a preheated incubator at 37°C for about 7-8 hrs. The contents were then removed from the semi-permeable membrane, 2 ml of 1 N sulphuric acid was added, and titrated with 0.05 N KMnO₄ until a light pink color endpoint was obtained. The amount of undissolved calcium oxalate was subtracted from the total quantity used in the experiment to determine the quantity of calcium oxalate that the test substance(s) could dissolve [16].



Fig. 4. *In vitro* Experimental set-up maintained at 37°C.

Statistical analysis

All values obtained from the experiment are presented as mean $\pm$ standard error of the mean. The values at different doses were compared statistically with controls using One Way ANOVA with the help of Graph pad statistical software [17].

Result

Phytochemical screening

The preliminary phytochemical investigation revealed the presence of alkaloids, saponins, flavonoids, and terpenoids in *I. psittacina*, saponins, flavonoids, steroids, and glycosides in *W. somnifera*, and alkaloids, tannins, flavonoids, terpenoids, and glycosides (table1) in *K. pinnata*, which may be responsible for the dissolution of calcium oxalate crystals.

Table 1. Phytochemical screening of *I. psittacina*, *W. somnifera*, and *K. pinnata*.

Test	Alkaloids	Flavonoids	Tannins	Terpenoids	Saponins	Steroids	Glycosides	Anthraquinone
<i>I. psittacina</i>	✓	✓	-	✓	✓	-	-	-
<i>W. somnifera</i>	-	✓	-	-	✓	✓	✓	-
<i>K. pinnata</i>	✓	✓	✓	✓	-	-	✓	-

Effect of plant extracts on the dissolution of calcium oxalate crystals using the titration method:

The effect of the extracts on calcium oxalate dissolution was determined using titration. The dissolution of calcium oxalate crystals was estimated in the presence of different plant extracts, without the extract, and along with the standard drug Cystone (control). The percentage of dissolution was then determined. All extracts showed different dissolution properties in a dose-dependent manner. But, the aqueous extract of *W. somnifera* showed significant dissolution of calcium oxalate crystals when compared with the other two plants *I. psittacina* and *K. pinnata*.

Table 2. Effect of aqueous plant extracts on calciumoxalate dissolution

Drug	Concentrations	Dissolution of CaOx crystals	Dissolution %
Cystone	10mg/ml	2.47 ± 0.06	82.33%
IPA	10mg/ml	1.32±0.07	44%
	20mg/ml	1.72±0.01	57.33%
	30mg/ml	2.31±0.03	77%
WSA	10mg/ml	1.67±0.26	55.66%
	20mg/ml	2.23±0.09	74.33%
	30mg/ml	2.25±0.07	75%
KPA	10mg/ml	1.45±0.02	48.33%
	20mg/ml	1.74±0.02	58%
	30mg/ml	2.38±0.01	79.33%

*IPA: *Impatiens psittacina* aqueous, WSA: *Withania somnifera* aqueous & KPA: *Kalanchoe pinnata* aqueous.

Table 3. Effect of methanolic plant extracts on calcium oxalate dissolution

Drug	Concentrations	Dissolution of CaOx crystals	Dissolution %
Cystone	10mg/ml	2.47 ± 0.06	82.33%
IPM	10mg/ml	1.45±0.02	48.33%
	20mg/ml	1.86±0.09	62%
	30mg/ml	2.33±0.02	77.66%
WSM	10mg/ml	1.99±0.22	66.33%
	20mg/ml	2.20±0.10	73.33%
	30mg/ml	2.44±0.09	81.33%
KPM	10mg/ml	1.51±0.03	50.33%
	20mg/ml	1.75±0.03	58.33%
	30mg/ml	2.42±0.06	80.66%

*IPM: *Impatiens psittacina* methanol, WSM: *Withania somnifera* methanol & KPM: *Kalanchoe pinnata* methanol.

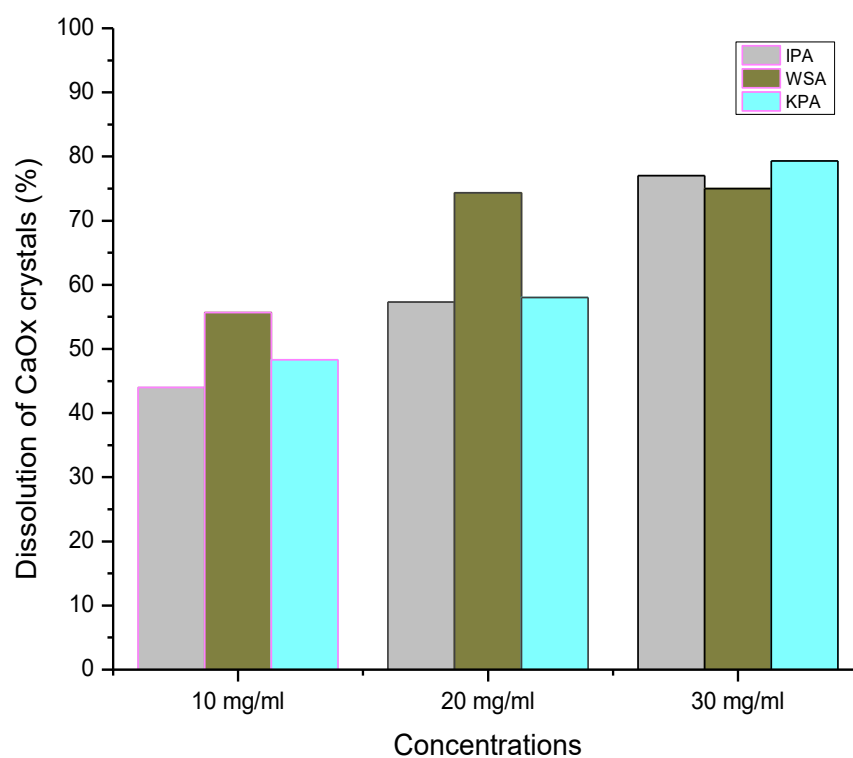


Fig. 5. Graph showing the dissolution percentage of CaOx crystals by aqueous extract of *I. psittacina*, *W. somnifera* and *K. pinnata*.

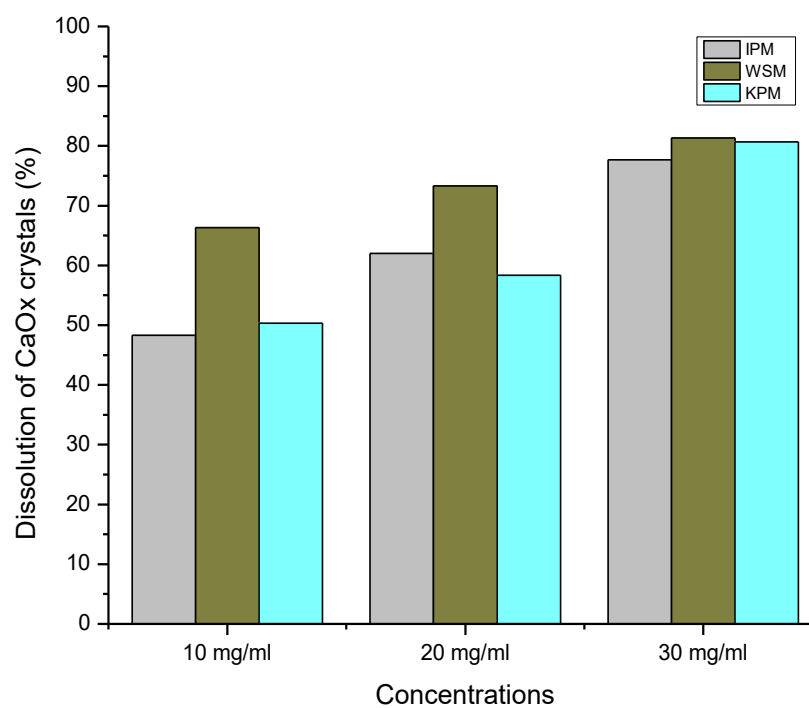


Fig. 6. Graph showing the dissolution percentage of CaOx crystals by methanolic extract of *I. psittacina*, *W. somnifera* and *K. pinnata*.

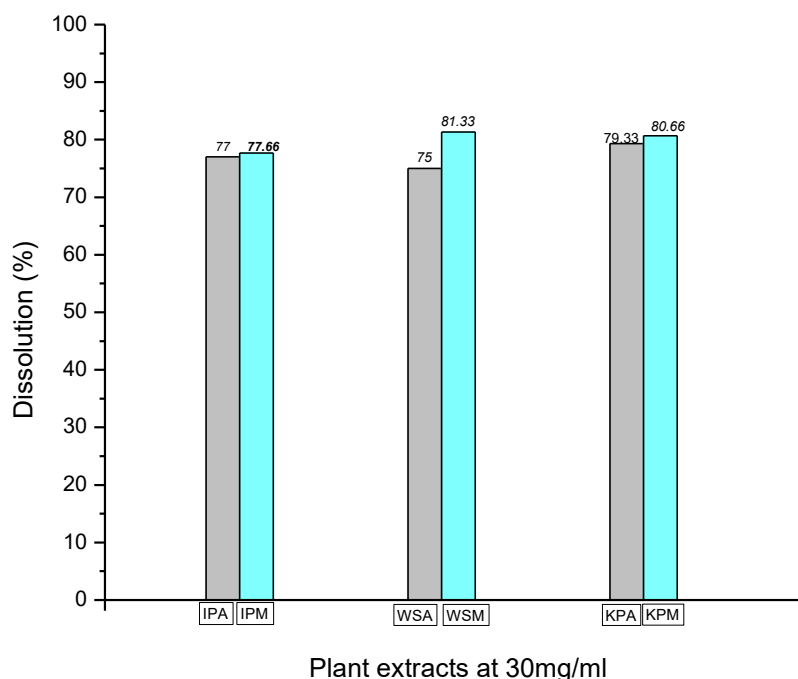


Fig.7. Graph showing the comparison of percent dissolution of both the extracts of *I. psittacina*, *W.somnifera* and *K. pinnata* at its highest concentration.

Discussion

There is growing evidence that calcium oxalate urolithiasis is associated with renal injury. A large amount of oxalate in the kidney may cause several changes in renal epithelial cells, such as an increase in free radical production and a decrease in antioxidant status, cell injury, and cell death. These are pre-responding factors that accelerate crystal adherence and retention [18]. *I. psittacina*, *W. somnifera*, and *K. pinnata* exhibit various medicinal properties, including antiurolithiatic activity. Phytochemical analysis of *I. psittacina* revealed the presence of alkaloids, saponins, flavonoids, and terpenoids. Likewise, *W. somnifera* contains saponins, flavonoids, steroids, and glycosides, and *K. pinnata* contains alkaloids, tannins, flavonoids, terpenoids, and glycosides (Table 1). *In vitro* analysis revealed that the methanolic fractions of all the plants showed the highest dissolution of CaOx crystals at 30 mg/ml, that is, the highest dose. The methanolic fraction of *W. somnifera* at 30 mg/ml concentration showed the highest dissolution of calcium oxalate of 2.44 ± 0.09 mg/ml i.e 81.33% dissolution property as compared to methanolic extract of *K. pinnata* (2.42 ± 0.06 mg/ml) and *I. psittacina* (2.33 ± 0.02 mg/ml) i.e 80.66% and 77.66% dissolution property respectively (Table 3). The aqueous fraction of *K. pinnata* at 30 mg/ml concentration showed the highest dissolution of calcium oxalate of 2.38 ± 0.01 mg/ml i.e 79.33% dissolution property as compared to aqueous extract of *W. somnifera* (2.25 ± 0.07 mg/ml) i.e 75% and *I. psittacina* (2.31 ± 0.03 mg/ml) i.e 77% of dissolution property (Table 2). These results were comparable with those of the positive control, which showed crystal dissolution at a concentration of 2.47 ± 0.06 mg/ml i.e 82.33% dissolution property. Thus, among the three plants, *W. somnifera* showed the highest efficacy against urolithiasis at its highest dose concentration (Fig.7).

Conclusion

Modern synthetic drugs are not only very efficient in curing a number of diseases but also cause serious adverse effects. Crude drugs are less efficient in curing the disease but are relatively free of side effects. Although the system of traditional medicine has enough strength and sustainability in its treatment, it is also observed that the system is not organized and is still practiced as an assortment of natural herbal medicine, superstition, and religious belief. Therefore, the exploration of this indigenous knowledge and its scientific

validation, recognition, and popularization in the system of medicine is urgently required to uphold this highly potential traditional healing practice of North East India. Incorporation of traditional medicines in a well-established system, such as Ayurveda and Unani, after progressing through various validation practices, such as phytochemical and pharmacological screenings of extracts and the isolation of active compounds due to the scarcity of marked drugs in the locality, may also be required to fulfill the need of time. Thus, several herbal plants or formulations have been evaluated for the treatment of calcium phosphate and calcium oxalate-type kidney stones. *In vitro* investigations of *I. psittacina*, *W. somnifera*, and *K. pinnata* have demonstrated antiurolithiatic activity against kidney stones. Such studies provide useful insights into the chemical profiles of plants and help validate folk medical knowledge and the capacity building of traditional healers. Many diseases can still be treated by certain plants and herbs from nature, and plants prescribed by traditional healers form a promising source for the selection of potential medicinal plants for validation and drug discovery.

Declaration

The authors declare no conflicts of interest. The authors did not receive any funding for carrying out the research.

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