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In-vitro Anti-Nephrolithiatic Activity of Selected Medicinal Plants

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ABSTRACT

Today, due to the changing lifestyle, a significant number of people are suffering from nephrolithiasis (kidney stones). Unfortunately, there is no satisfactory medication for use in clinical treatment. The current study is an ethno-pharmacological study aimed at *in vitro* evaluation of the anti-nephrolithiatic assessment of some selected medicinal plants that are traditionally used for nephrolithiasis treatment. An *in vitro* study was conducted to assess the anti-nephrolithiatic activity of aqueous plant extracts (*Paronychia argentea*, *Teucrium polium*, *Alhagi maurorum*, *Crataegus aronia*, *Varthemia iphionoides*) by evaluating inhibition of calcium oxalate (CaOx) nucleation and aggregation. All tested plant extracts showed nucleation inhibition in a dose in-dependent manner. *V. iphionoides* showed maximum aggregation inhibition when used at a low concentration. Our findings support the ethno-pharmacological use of these plants in the prevention or treatment of kidney stones.

Keywords: Nephrolithiasis, CaOx stones, Neucleation, Inhibition, Aggregation, Plant extracts

Introduction

Nephrolithiasis, or kidney stone disease, is an ailment in which individuals form calculi (stones) within the renal pelvis and tubular lumens.[1] Long-term pharmacological treatment is essential to reduce kidney stone recurrence in the population. Furthermore, the field of kidney stones pharmacological treatment is deficient in new agent testing because of the lack of availability of concisely measured outcomes.[2] The World Health Organization (WHO) reported that about three-quarters of the world's population rely on traditional medicine, particularly herbal medicine. In recent years, the use of herbal drugs from ethno-medicine has increased the attention in ethno-botanical studies throughout the world.[3] We made a list of medicinal plants which have been utilized for the treatment of kidney stones in Jordanian ethno-pharmacological; such as: *Paronychia argentea* lam, *Teucrium polium* L., *Alhagi maurorum* Medik., *Varthemia iphionoides* A.P, *Crataegus aronia* L. *P. argentea* known as "Rejel El-Hamama" in Arabic (Caryophyllaceae) is an annual species, 30 cm in height, elliptical and mucronate leaves, lateral and terminal flowers emerge in glomerulus [4]. *P. argentea* is extensively used in folk medicine for the treatment of many conditions such as kidney stones, urinary tract infections, heart disease, diabetes in addition to gastrointestinal conditions.[5,6] *T. polium* known as "Jaa'deh" in Arabic (Labiatae), is a dwarf, pubescent, aromatic shrub, oval leaves with enrolled margins and dense heads of white flowers. *T. polium* is an edible plant that is also commonly used in traditional medicine. It is one of the most prescribed plants by the herbalist for abdominal colic, headache, antispasmodic, antidiabetic, hypertension, flatulence and to treat kidney stones. [6-8]

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A. maurorum known as "Aqool" or "Shook El-Jamal" in Arabic (Fabaceae). *A. maurorum* is a promising medicinal plant with a wide variety of pharmacological activities that can be used in many medical applications. It has had antibacterial, anti-inflammatory, antipyretic, analgesic, antioxidant, neurological, cardiovascular and dermatological impact and several others.[8] *V. iphionoides* (synonym for *Chiliadenus iphionoides*) known as "Ktilih" or "Shtilih in Arabic (Compositae). *V. iphionoides* is used in traditional medicine to relieve pain, gastrointestinal disorders, diabetes mellitus, heal wounds and urine retention.[9] *C. aronia* (synonym for *Crataegus Azarolus*) commonly called hawthorn in English and known as"Zaroor" in Arabic (Rosaceae).[8]*C. aronia* is commonly used in Arabic traditional medicine to treat cardiovascular diseases, hyperlipidemia, diabetes, and sexual impotence. Several beneficial effects for *C. aronia* were reported. [10, 11]
To the best of our knowledge, there is no comparable study that evaluates the anti-nephrolithiatic activity of the selected plant extracts in the literature, and all of the selected plants are considered culturally important in Jordan. Additionally, it is the first study to examine *P. argentea* water extract activity.
Thus, this study aimed to evaluate the *in-vitro* anti-nephrolithiatic assessment of *A. maurorum*, *C. aronia*, *P. argentea*, *T. polium*, and *V. iphionoides*.

Materials and Methods

Plant material
In Mafraq and Ajloun, Jordan, between May and July 2019, samples of A. maurorum roots, as well as V. iphionoides leaves and stems, were gathered. A local traditional herbal market in Amman, Jordan, was where aerial parts of *P. argentea*, *T. polium*, and leaves of *C. aronia* were purchased in April 2019. The plants were taxonomically identified with the assistance of Prof. Sawsan Oran, Department of Biological Science, Faculty of Science, University of Jordan, by direct comparison with confirmed samples in the Faculty of Science, University of Jordan, herbarium. The Department of Pharmaceutical Sciences at the University of Jordan's School of Pharmacy received the voucher specimens (Nos. ALHG- 2019, VAR- 2019, PAR- 2019, TAC- 2019, and GRAT- 2019). (Phytochemistry lab).

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Preparation of medicinal plant extracts
To mimic as closely as possible the most commonly used methods by laymen, maceration was followed by decoction. Thirty grams of each finely ground plant material was first soaked in 500 ml of distilled water, mixed and left to stand for 24 hour with continuous shaking. Then they were boiled for 15 min. at a100°C mixture, filtered and lyophilized. Plant extracts were kept in sterile containers and stored at -4 °C until required.

In vitro anti-nephrolithiatic activity of plant extracts
Turbidometric method: The in vitro anti-nephrolithiatic activity of aqueous plant extracts of (*P. argentea*, *T. polium*, *A. maurorum*, *V. iphionoides*, and *C. aronia*) has been studied in terms of inhibition of CaOx nucleation and aggregation in the presence of inhibitors (extracts and standard drug) and absence of inhibitors. A UV-Visible spectrophotometer was used in order to measure the turbidity changes in each assay. Three different concentrations (10 mg/mL, 20 mg/mL and 30 mg/mL) of the plant extracts were tested in each assay.[12]

Nucleation assay
Solutions of calcium chloride ($CaCl_2$) and sodium oxalate ($Na_2C_2O_4$) were prepared at the final concentrations of (5 mmol/L) and (7.5 mmol/L) correspondingly in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. At various concentrations (10 mg/mL, 20 mg/mL, and 30 mg/mL), 950 µL of $CaCl_2$ solution was combined with 100 µL of plant extracts, fractions and the standard drug. Crystallization was started by adding 950 µL of $Na_2C_2O_4$ solution and the temperature was sustained at 37°C. The growth of crystals was predictable due to the following reaction:

$$CaCl_2 + Na_2C_2O_4 \longrightarrow CaC_2O_4 + 2NaCl$$

The absorbance of the solution was measured at 620nm. The nucleation was estimated by comparing the absorbance with that of control in the presence of the extract.
% inhibition of nucleation was calculated using the following formula:

% Inhibition of nucleation = [(C-S)/C] × 100
Where, C is the turbidity without extract and S is the turbidity with extract.[13]

Aggregation assay
The effect of plant extracts and fractions on CaOx crystal aggregation was determined by means of aggregation assay. Solutions of $CaCl_2$ and $Na_2C_2O_4$ (50 mmol/L each) were mixed together, heated in a water bath for 1hour at 60°C and then incubated overnight at 37°C to prepare seed crystals of CaOx. When dried, a (0.05 mol/L) Tris-HCl and (0.15 mol/L) NaCl at pH 6.5 buffer. A CaOx crystal solution (0.8 mg/mL) was prepared. One milliliter of aliquots (10mg/mL, 20mg/mL and 30mg/mL) of plant extracts and fractions were added to a 3 mL CaOx solution and then vortexed. The absorbance of the final mixtures was then read at 620nm wavelength and the % inhibition of aggregation was then calculated as described in nucleation assay.[12] The samples attained from nucleation and aggregation assays were observed under a light microscope equipped with a digital camera to visualize the CaOx crystal formation.

Qualitative phytochemical screening
Preliminary phytochemical screening for the presence of flavonoids, tannins, terpenes, saponins and alkaloids was performed using standard.[13]

Results and Discussion

The nucleation rate was assessed by measuring the alteration in turbidity in the presence of an extract, using an extract-free as control. The aqueous plant extracts and Cystone® inhibited CaOx nucleation in a dose in-dependent manner. The maximum inhibition percentage was observed at 10 mg/mL concentration in *P. argentea* (74%), *T. polium* (64%), *V. iphionoides* (60%) and *C. aronia* (68%). However, the maximum percentage inhibition was observed at 20 mg/mL concentration in *A. maurorum* (40%) and Cystone® (65%)**.** Similarly, the aggregation rate was assessed by measuring the alteration in turbidity in the presence of the extract. *V. iphionoides*, *A. maurorum*, *C. aronia* and Cystone® inhibited CaOx aggregation in a dose in-dependent manner. The maximum percentage inhibition was observed at 10 mg/mL concentration in *V. iphionoides* (74%) and *C. aronia* (47%). While, the maximum percentage was observed at 20 mg/ml concentration in *A. maurorum* (38%) and Cystone® (50%). The aggregation percentage inhibition of *P. argentea* ranged from (-178% to 3%) and *T. polium* ranged from (-3% to7.5%). Table 1. The results of the phytochemical screening of aqueous plant extract are presented in Table 2. CaOx nephrolithiasis is the most predominant type of kidney stones compared to other kinds of kidney stones illnesses[14, 15]. The effectiveness of aerial parts of *P. argentea* and *T. polium*, roots of *A. maurorum*, leaves of *C. aronia* aqueous extracts and leaves and stems of *V. iphionoides* aqueous extract against CaOx nephrolithiasis was demonstrated in the present study. Our emphasis was especially focused on phytotherapy, which is common in traditional medicine as an alternative to primary healthcare in numerous countries. Therefore, the studied plants broadly distributed in the Mediterranean area, are used in traditional medicine to treat kidney stone. However, pharmacological studies about the effectiveness of these plants and supporting folk information regarding their anti-nephrolithiatic activity are rare. Nucleation, is the important phase in the stone forming process. The formation of CaOx crystals results in the incubation of a metastable solution of $CaCl_2$ and $Na_2C_2O_4$.[16] Crystal formation is not as important as crystal retention. Aggregation plays the main role in crystal retention. It marks the process where several crystals in the solution come together and adhere forming large crystal agglomerates.[17] It occurs very promptly and exerts a great effect on particle size.[18] It was discovered that all plant extracts inhibited CaOx nucleation and aggregation in a dose in-dependent manner. These results were in agreement with other previous studies which showed that CaOx nucleation and aggregation were inhibited in a dose-independent.[13,19]

Table 1: Percentage inhibition of nucleation and aggregation for CaOx by aqueous plant extracts and Cystone®

Plant extract	Concentration	% inhibition of nucleation	% inhibition of aggregation
P. argentea	10 mg/mL	72	3
	20 mg/mL	70	-50
	30 mg/mL	54	-177
T. polium	10 mg/mL	64	7
	20 mg/ml	40	3
	30 mg/mL	54	2
V. iphionoides	10 mg/mL	60	74
	20 mg/mL	40	48
	30 mg/mL	52	53
A. maurorum	10 mg/mL	34	47
	20 mg/mL	40	48
	30mg/ml	36	94
C. aronia	10 mg/mL	48	42
	20 mg/mL	46	38
	30 mg/mL	26	17
Cystone®	10 mg/mL	58	32
	20 mg/mL	65	50
	30 mg/mL	41	31

Table 2: Preliminary phytochemical screening

	Flavonoids	Tannins	Saponins	Terpenoids	Alkaloids
*P. argentea (*aerial parts)	+	-	-	-	-
*T. polium (*aerial parts)	+	+	+	-	-
V. iphionoides (stem &leaves)	+	+	-	-	-
A. maurorum (roots)	-	-	+	-	-
C. aronia (leaves)	+	-	-	+	-

+Present,-Absent

A possible explanation for that might be related to CaOx polymorphism phenomenon when CaOx monohydrate, thermodynamically extra stable crystals with more adhesive affinity, loose their crystalline nature and convert to CaOx dihydrate which considered as a critical step in the inhibition of stone formation.[12] This phenomenon possibly occurred due to reduced surface free energy by some phytochemicals found in plant extracts. [20]However, further investigations are necessary, to provide the mechanism, using available methods of identification of CaOx crystal shape such as X-ray diffraction, infrared spectroscopy, scanning electron microscopy and Raman microprobe analysis.[18,21]
V. iphionoides aqueous extract showed the maximum inhibitory effect on crystals aggregation. However, an increased aggregation rate was seen when *P. argentea* and *A. maurorum* were used at higher concentrations. These results might be caused by oxalate present in these plants which might promote crystallization, especially at higher doses. Oxalate is an organic chemical that is produced by plants to help them remove unwanted calcium from the local groundwater absorbed by their roots. One study showed the abundance of oxalate in several Crataegus species leaves which reached about 28%.[22] Unfortunately, there is no previous data reported on the percentage of oxalate in the plants used in our study.

Conclusion

Our results support the ethno-pharmacological use of *P. argentea*, *T. polium*, *V. iphionoides*, *A. maurorum* and *C. aronia* in the prevention or treatment of kidney stones. Thus support the significance of medicinal plants in current medicine and drug discovery.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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