In Vitro Study of the Litholytic Effects of *Zilla Spinosa* L. and *Leuzea Conifera* L. on Urinary Cystine Stones

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***Abstract*—** Aim: The present study aimed to evaluate and compare the litholytic effect of two medicinal species used in Algerian traditional medicine to treat the cystine stones: *Zilla spinosa* L. (leaves and roots), and *Leuzea conifera* L. (roots).

Material and methods: Aqueous extracts of the two plants were prepared and tested for their litholytic effect against cystine stones collected from different hospitals in East Algeria. Cystine stones of comparable masses and identical morphology were maintained in the aqueous extracts for eight weeks. NaCl (9g/L) was used as a control. Mass of the stones was measured every 15 days and pH of the solutions every 3 days.

Results: Our results showed a marked litholytic effect of both extracts. Indeed, *L. conifera* and *Z. spinosa* extracts resulted in a mass loss of 103.34±36.6 and 98.05±15.94 mg, respectively, whereas that of the control was found to be 40.6±15.08 mg. On the other hand, pH values were maintained at 5.8 and 6.1.

Conclusion: The present study demonstrated that *L. conifera* and *Z. spinosa* aqueous extracts exhibited an important litholytic effect. Therefore, these plants could be promising sources of litholytic molecules.

***Index Terms*—** Cystine stones, *Leuzea conifera L.*, litholytic effect, *Zilla spinosa L.*,

I. Introduction

Nowadays, medicinal plants are used by more than 80% of African local populations to treat and/or prevent different diseases (WHO, 2002). Nonetheless, the use of such species without a scientific basis could generate dangerous adverse effects and may represent a real public health issue. Algerian populations still use different medicinal species as alternative treatments against health problems such as cancer (Benarba et al., 2014) or urolithiasis.

Urolithiasis is the formation of urinary calculi (stones) in the urinary system. These stones are characterized by different structures and chemical composition, and result from different factors such as genetic factors, metabolism, diet, and water intake (Ravichandiran et al., 2017). Most of the urinary calculi are oxalocalcic stones, in which the calcium oxalate is found in the form of monohydrate ($CaC2O4{\cdot}H2O$), dehydrate ($CaC2O4{\cdot}2H2O$) or a combination of both (Alelign et al., 2018). Although the use of medicinal species to treat this form of lithiasis was studied in different countries (Kachkoul et al, 2018; Bouanani et al., 2010; Meiouet et al., 2011), few previous works have been carried out regarding cystine lithiasis which accounts for 1% of the stones in adults and 10% in children (Lechevallier et al., 2008).

Cystinic lithiasis is the result of an inherited abnormality of renal transport (proximal convoluted tubule) of cystine and dibasic amino acids (ornithine, arginine and lysine). The defect of tubular reabsorption induces an abundant urinary excretion of these amino acids. Since cystine is low soluble in acidic and neutral urine, its excretion results in the formation of urinary cystine stones (Fattah et al, 2014; Traxer et al., 2008). The treatment of cystine stones must include both the treatment of the stones and their complications, and the prevention of recurrence. It therefore depends on a good knowledge of the natural history of this disease and of the different therapeutic possibilities available (Shim and Park, 2014). Unfortunately, despite considerable progress in the medical field, the therapy of this physico-chemical variety remains difficult at present. In addition to the repeated surgical interventions induced by a lack of early diagnosis, cystine stones are indeed particularly resistant to extracorporeal shock waves and almost inaccessible to pulsed dye laser (504 nm) (Shim and Park, 2014).

Medicinal plants represent a promising source of alternative molecules with anti-lithiasic properties. Indeed, several ethnobotanical studies have identified different medicinal species used by local populations to treat urolithiasis such as *Parietaria officinalis* L. and *Erica arborea* L. in Algeria (Benarba et al., 2015), *Alhagi maurorum* L. and *Tribulus terrestris* L. in Iran (Bahmani et al., 2016), or *Dolichos biflorus* L. in India (Yadav et al, 2011). Moreover, the antilithiasic effects of different medicinal plants have been demonstrated both in vitro and in vivo (Akram and Idrees, 2019; Yachi et al, 2018). Nevertheless, these studies are almost exclusively oriented towards calcium lithiasis (Atmani and Khan, 2000; Djaroud et al., 2012; Charafi et al., 2012).

The present study aimed to evaluate the in vitro effect of two Algerian medicinal plants: *Zilla spinosa* L. (Bassicaceae) and *Leuzea conifer* L. (Asteraceae) on the dissolution of cystine kidney stones.

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II. MATERIALS AND METHODS

A. Vegetal Material

Two medicinal plants of the Algerian flora represented in fig 01 (a) and (b) and Table □, are the subject of this research. Roots and leaves of *Z. spinosa* were harvested in December 2014 in Tamanrasset, Southern Algeria. The roots of *L. conifera* were collected in the region of Taoura, Eastern Algeria, in April 2014. Both plants were botanically identified by a pharmacist botanist from the Faculty of Medicine of Annaba, Algeria.

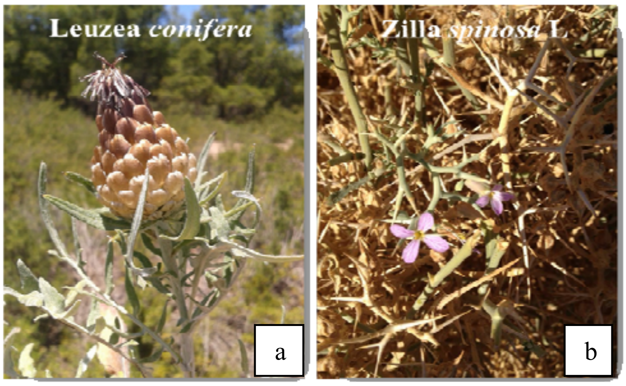

Fig 1: *Leuzea conifera* L (a) and *Zilla spinosa* L. (b) (Photos taken by A. Lekouaghet A and R. Mecheri , 2014)

TABLE □: Monograph Of The Two Plants Studied.

Scientific name	Vernacular name	Family	Way of use	Used Parts
***Zilla spinosa* L**	Aftezzen	Brassicaceae	Infusion	Air parts
***Leuzea conifer* L**	El kaze	Asteraceae	Maceration	Roots

B. Extraction

The extract of L. conifer roots was prepared by macerating 10 g of the dried roots in 100 ml of an aqueous solution of sodium chloride (NaCl) at 9 g/l, for 12 hours. The extract of Z. spinosa was prepared by infusion of 10 g in 100 ml of sodium chloride solution (NaCl) at 9 g/L previously boiled for 30 minutes. The extracts were then filtered.

C. Phytochemical Screening

The chemical screening carried out allows the detection of the different families of secondary metabolites existing in the studied part of the plant by qualitative characterization reactions based on precipitation or staining phenomena by reagents specific to each family of compounds as well as examinations by ultraviolet light (Bruneton, 2003).

D. Dissolution Test of Cystine Stones

Nine pure cystine kidney stones of subtype Va (Benarba et al, 2014) , of similar dimensions from the laboratory of the Faculty of Medicine of Annaba (Algeria) were tested (Fig 02). Their average size and mass are respectively 4 mm and 183.78±64.67mg.

The prepared solutions (extract and control solution of NaCl) were distributed in fractions of 30 ml in beakers containing an equal volume of phosphate buffer (Na2HPO4 at M/15 and KH2 PO4 at M/15, pH = 6).

Each calculus was placed in a porous bag of braided fibers to avoid any contact with the container's wall. The extracts and NaCl control solution were changed every 3 days, after measuring their pH. The experiment was carried out in total for eight cumulative weeks, at room temperature under stirring in a shaker.

The weighing of the calculi was carried out using a precision balance (Schimatzu AUW 220 OD) every two weeks after being rinsed with distilled water, and dried for 18 hours at 40 ° C. For each type of extract, the experiments were repeated with six stones to determine the mass and pH averages. The dissolution rate of the calculi was determined using the following formula:

$$a\% = \frac{(m_i - m_f)}{mi}.100$$

a%: Stone dissolution rate

m_i and **m_f:** Average mass of calculi before and after treatment

The experimental and measurement protocol, carried out for plant extracts, was rigorously followed for a sodium chloride (NaCl) solution at 9 g/L considered as control.

Fig 2 Cystine stones subtype Va

E. Micrographic Observations

The micrographic observations were carried out using a JEOL JSM-7100F field scanning electron microscope (SEM) at 2kV voltage.

F. Statistical analysis

Statistical analysis was performed using Minitab 16. The results are expressed as mean ± SD.

The statistical differences were evaluated by one-way ANOVA followed by Tukey test, and statistical significance was defined as $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Results

Phytochemical Screening

As shown in TABLE □, different phytochemical compounds were detected in the roots of L. coniferea, and leaves of Z. spiosa. Indeed, both aqueous extracts contain polyphenols, flavonoids, sterols, polyterpenes, and saponins.

TABLE □: PHYTOCHEMICAL SCREENING OF AQUEOUS EXTRACTS OF *ZILLA SPINOSA* L. AND *LEUZEA CONIFERA* L.

		Chemical groups							
Species	Plant part	Sterols and polyterpens	Polyphenols	Tanins		Flavonoids	Saponins	Coumarins	Alkaloids
				gallic	catechic				
Z. spinosa	Aerial parts	+	+	+	-	+	+	-	+
L. conifera	Roots	+	+	-	+	+	+	+	-

(-) : Absence ; (+) : Presence

Our results showed that alkaloids and gallic tannins were present in *Z. spinosa* extract and absent in that of *L. conifera*, whereas catechic tannins and coumarins were detected only in *L. confiera*.

B. Dissolution of Cystine Stones:

Our results showed that exposure of cystine stones to *L. confiera* and *Z. spinosa* aqueous extracts was associated with a significant mass loss of 103.34 ± 36.6 mg and 98.05 ± 15.94 mg respectively. However, the mass loss of cystine stones treated with NaCl was found to be versus 40.6 ± 15.08 mg. Fig 3a shows the evolution of stones mass during the 8 weeks of experiment highlighting a progressive and sustained mass loss of cystine stones treated with the two plant extracts.

As illustrated in Fig 3b, the dissolution rate of cystine stones increased in a significant time-dependent manner. The dissolution rates were 66.7%, 63.4% and 23% for the cystine stones exposed to L. conifera, Z. spinosa, and NaCl, respectively**.**

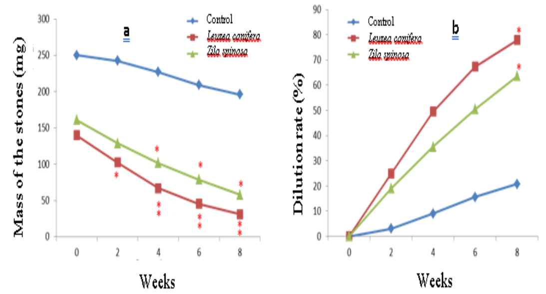

Fig 3: Evolution of mass (a) and mass loss rate (b) as a function of holding time in extracts of *Leuzea conifera* L. (100g/l), *Zilla spinosa* L. (100g/l) and control NaCl solution (9g/l).

*: Significant difference comparing to control (P ≤0.05).
**: Highly significant difference comparing to control (P ≤0.01).

C. pH evolution :

TABLE □ shows the evolution of pH values during the eight weeks. Measurements were performed every three days. According to the results obtained, there were no significant changes of pH values following the exposition of cystine stones to both extracts.

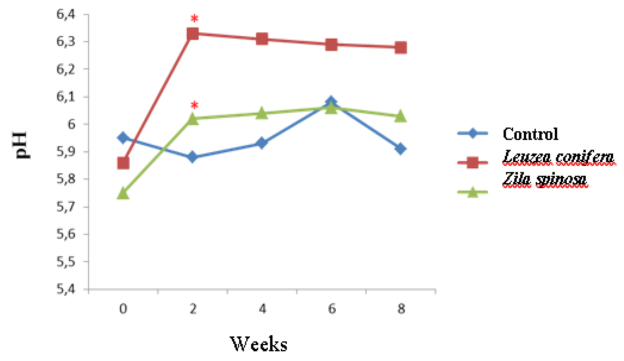

TABLE □: pH Values During The Eight Weeks Of Experimentation

Weeks / Days	Two weeks				Four weeks					Six weeks					Eight weeks				
	3	6	9	15	18	21	24	27	30	33	36	39	42	45	48	51	54	57	60
Control solution	5,84	5,85	5,90	5,88	5,9	5,9	5,9	5,9	5,9	5,9	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8	5,8
	5,87				5,93					5,84					5,81				
Z. pinosa	6,05	5,94	6,03	6,04	6,1	6,1	5,9	5,9	6,0	6,1	6,1	6,0	6,0	6,0	6,0	6,0	5,9	5,9	6,0
	6,02				6,04					6,05					6,03				
L. conifera L	6,13	5,95	6,02	6,12	6,0	6,1	6,1	6,1	6,1	6,1	6,1	6,0	6,1	6,0	6,1	6,1	6,1	6,1	6,1
	6,06				6,11					6,09					6,13				

In **fig 4** is shown the evolution of the pH of the three solutions as a function of the residence time of the cystinic stones. After a slight rise, recorded just two weeks after the maintenance, the pH stabilizes for both plant extracts, remaining below 6.38 for Leuzea conifera L and 6.03 for Zilla spinosa L. For the control solution, the pH fluctuates only slightly, varying between 5.86 and 6.08.

Fig 4: pH evolution of the two extracts and the control solution during the cystine stone holding stay.

*: Significant difference comparing to control (P ≤0.05).
P: Threshold of significance.

D. *Micrographic Observations:*

The Scanning electron microscopy of cystine stones before and after eight weeks exposition is given in Figure 5. As can be seen from figure (5a, 5b), cystine stones showed a well-arranged crystallite surface, resulting in a surface compact appearance where crystallites have hexagonal-shaped facies, a feature typically observed in cystine stones at this scale. Growth twins also have a hexagonal shape with very straight edges.

Regarding the effect of NaCl solution, our results showed that the stones surfaces experienced a partial degradation and appeared with less relief. Nonetheless, stones dissolution was found since there was a consistent loss of the original hexagonal form of the cystine crystallites (Figs.5c-5d).

In fact, for Z. spinosa extract, stones surface showed an overall appearance in sheets, reflecting strong erosion, with a total loss of the initial hexagonal form of the cystine facies (Figs. 5e-5f).

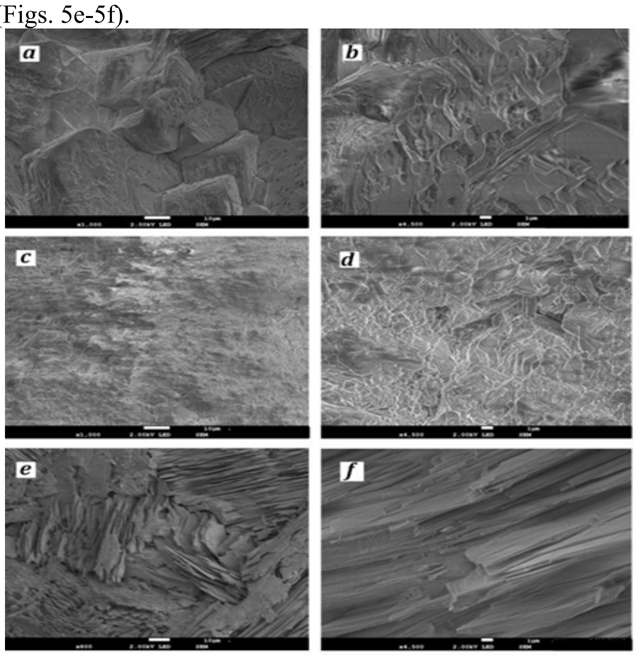

Fig 5: SEM micrographs of the surface condition of cystine stones: (a, b) before treatment, (c, d) after eight weeks in aqueous NaCl solution, (e, f) after eight weeks in *Zilla spinosa* L. extract.

IV. DISCUSSION

The use of medicinal plants to treat urolithiasis is common among the Algerian population. In fact, several ethnobotanical studies reported various local medicinal species used for the treatment of this pathology (Hammiche et al., 2006; Khitri et al., 2016; Sekkoum, 2011).

The main objective of the present study was to evaluate the in vitro litholytic effect of two medicinal species: *Z. spinosa* and *L conifera*. The preparation methods and doses were similar to those currently practiced in Algerian pharmacopeia.

Our results showed that the cystine stones mass loss was significantly higher with plants extracts (65%) than with NaCl control (23%). This dissolution was carried out in the presence of a phosphate buffer ensuring the extracts a slightly acidic pH, between 5.8 and 6.1. This finding is interesting since cystine is poorly soluble at these pH values, and its solubility limit does not exceed 1.2 mmol/l for pH <6.5 (Fig. 4). Cystine stones dissolution, as reported by similar studies (Meiouet et al., 2011), achieved without presence of a buffer allowing a free pH evolution to high values (Hannache et al., 2012). Accordingly, the stones mass loss was favored in several experiments by the medium alkalinization. When the two species were compared, no significant differences were observed ($p = 0.44$).

The mesoscopic scale-microscopic observations revealed that Z. spinosa extract resulted in an erosion of the stones surface, attributed therefore to their inter-interaction (with the extract). These findings are consistent with those previously reported by Hannache et al (2012). Indeed, they demonstrated that cystine stones treated with Arenaria ammophila and Parietaria officinalis showed surface erosion, accompanied by partial or total loss of the hexagonal shape of cystine crystallites, and presence of leaves separated by more or less wide spaces (hannache et al., 2012). Similar characteristics (surface erosion, structure in leaves and loss of the typical hexagonal shape) were found in cystine stones of patients receiving sulfhydryl drugs bearing thiol (bazin et al., 2014). Although a classical alkalinization such as trometamol could reduce the cystine crystallites size, it does not modify the crystals shape and it can generate the carbapatite formation (bazin et al., 2014; bazin, 2010).

The litholytic effect exerted by both *Z. spinosa* and *L conifer* extracts could be attributed to their active phytochemical compounds since the pH values remained relatively unchanged. These active compounds can form "cystine-active ingredient" complexes more soluble than cystine inducing therefore a higher dissolution. Indeed, the phytochemical screening of the two species revealed the presence of several bioactive compounds. Aerial parts of *Z. spinoa* were found to contain coumarins, sterols, triterpenes, polyphenols, triterpene saponins. Moreover, seven flavonoids types were isolated from these plant part (El-Sharabasy et al., 2012). On the other hand, different phytochemicals were found in *L. conifer* such as inulin-type polysaccharides in addition to condensed tannins, saponins, phenols, polyphenols and a high concentration of flavonoids (Atmani and Khan, 2000).

Thus, the "cystine-active ingredient" complexes formed during the eight-week present study might be of cystine-flavonoids, cystine-tannins or cystine-saponosides types. This process could be enhanced by hydrogen, and hydrophilic bonds between the functional groups of the active phytochemicals, and the cystine carboxylic or amine functions as suggested by Meiouet et al (2011). The nature and abundance of these compounds vary from one plant to another, which may explain the observed differences in efficacy. A synergistic process involving many of the complexes evoked can also occur and allow a more efficient cystine dissolution.

V. CONCLUSION

In the present study, we demonstrate an important in vitro urolitholytic effect of *Z. spinosa* and *L. conifera* aqueous extracts in the presence of a phosphate buffer (pH = 6). Both extracts resulted in significant mass losses of cystine stones demonstrating the efficiency of these species in the dissolution of this type of calculus in a slightly acid environment. This uro-litholytic effect could be attributed to the formation of active phytochemicals-cystine complexes.

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Mecheri Rim, Boutefnouchet Abdelatif, Smati Dalila, Daudon Michel. Evaluation of the antilithiasic, cystinic and oxalo-calcic effect of aqueous extract of Zilla spinosa L. 2023. ffhal-03952113f DOI : https://hal.science/hal-03952113
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