IJPSR (2024), Volume 15, Issue 3 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 July 2023; received in revised form, 22 November 2023; accepted, 30 December 2023; published 01 March 2024

IN-VITRO INHIBITION AND DISSOLUTION OF CALCIUM OXALATE STONES BY THE FRACTIONS OF THE *ZIZIPHUS LOTUS* L. LEAVES

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Keywords:

Antiurolithiatic activity, *Ziziphus lotus*, Polyphenols fraction, Flavonoids fraction, Alkaloids fraction, Calcium oxalate

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ABSTRACT: Nowadays, many clinical studies have provided reliable results on the effects of several herbal medicines on many diseases such as kidney problems. The objective of this study is to evaluate *in-vitro* the antiurolithiatic activity of polyphenols, flavonoids, and alkaloids fractions of the leaves of the species *Ziziphus lotus*. The quantification of the polyphenol and flavonoid fractions was carried out by spectrophotometric methods; on the other hand, the alkaloid fraction is quantified gravimetrically. The antiurolithiatic activity of the fractions is studied by the use of the turbidimetric and gravimetric techniques. The polyphenol content varies between 1198,696 ± 99,848 and 15270,751 ± 290,419 (µg GAE)/g). While flavonoids range from 97,340 ± 8,437 to 1745,864 ± 8,690 (µgQE/g). On the other hand, the alkaloid fraction represents 1. 26%. The polyphenols fraction showed a higher inhibition 89.80 ± 0.69 than the two other fractions which represent the values 84.17 ± 1.11 and 78.90 ± 1.40, respectively. The polyphenols fraction (AF: 86.66 ± 1.43) and flavonoids (DMF: 80.85 ± 2.03) show an increasing evolution of the capacity to dissolve the precipitate of calcium oxalate compared to the reference solution (Sodium citrate: 75.38 ± 2.03). On the other hand, the alkaloid fraction represents a less significant dissolving power (AlF: 66.81 ± 4.13). The antiurolithiatic activity of *Ziziphus lotus* leaves is due to the presence of bioactive compounds such as polyphenols, flavonoids, and alkaloids, these results deserve further study to isolate the active substances responsible for the inhibition and dissolution of crystals.

INTRODUCTION: Several drugs have been isolated from medicinal plants, most of them due to their use in traditional medicine. In recent years, more than two-thirds of the world's population has become dependent on plant-derived drugs [1]. The genus Ziziphus belongs to the family Rhamnaceae and is represented by 135-170 species [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.15(3).844-53

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.15(3).844-53

The specie *Ziziphus lotus* (*Z lotus*) known by the vernacular names 'Sedra', 'Nbeg' [3], generally, grows in arid or semi-arid countries [4].

In the last few years, several studies of this plant show that the pulp exerts significant antiobesity, hypoglycemic, antioxidant, and immuno-modulatory activities, other studies show that this species possesses anticancer, antifungal, antibacterial, antiulcer, and wound-healing properties [5, 6, 7]. The aqueous extracts have activities cytotoxicity and anti-inflammatory [8], on the other hand, the extract of this plant is used for their effect antifungal [9]. *Z. lotus* leaves contain

bioactive compounds, such as polyphenols (Resveratrol, Pyrogallol, Gallic acid), phenolic acids (Chlorogenic, p-Hydroxybenzoic, Caffeic, Vanillic, Syringic, p-Coumaric, Ferulic, Sinapic, Rosmarinic), flavonoids (Rutin Luteolin, kaempferol, Apigenin, Quercetin, Naringin, Epicatechin), tannins and saponins (jujuboside B, jujubogenin glycosides, jujubasaponine IV) [1, 6, 7, 10]. The extracts of *Z. lotus* also showed the contents of alkaloids, flavonoids, anthraquinones, and tannins [11]. Several phytochemical families, found in most herbal remedies such as phenolic compounds, under physiological conditions, these phenolic compounds can also form phenolate ions [12]. Plants of the Rhamnaceae family are the source of cyclopeptide alkaloids [13], and according to the research [14] the alkaloids isolated from the *Z. lotus* root are Lotusines, on the other hand, the aerial parts of the plant also have other alkaloids, like Lotusanine A, B, Sanjoinenine, Sanjoinine F and Frangufoline [15, 16].

Cyclopeptide alkaloids of Ziziphus species showed interesting biological properties, including, sedative, analgesic, and immunostimulant [13, 17]. Researchers use alkaloids in the urolithiasis field, for example, berberine which is an alkaloid present in many medicinal plants and used in the prevention of urolithiasis [18]. Nowadays phenolic compounds, alkaloids, saponins, lignins, glycosides, and terpenoids have become a part integral to modern medicine [19]. The type of stones depends on the composition of the urine and the nature of the diet consumed by the population [20]. Calcium oxalate (CaOx) represents up to 80% of the stones analyzed and generally exists in different forms: calcium oxalate monohydrate (COM), dihydrate (COD), and rare trihydrate (COT) [21, 22].

The crystallization of stones starts with crystalline particles in the urethra, followed by a nucleation step. These crystals will then grow and aggregate with other crystals in the solution, and accumulate in the kidneys [23]. Historically, medicinal plants have been used as therapeutic remedies due to the presence of bioactive antiurolithiatic compounds such as phenolic compounds, saponins (solasodine), flavonoids (quercetin, kaempferol, luteolin), alkaloids (berberine), tannins, furanochromones (Khellin, Visnagin) and protein plants [19]. The present study aims to investigate *in-vitro* the antilithiatic ability of polyphenols, flavonoids, and alkaloids fraction of *Z. lotus*, on urolithiasis of the Calcium oxalate dihydrate (COD) type, all this to evaluate potential therapeutic use to treat and/or to prevent nephrolithiasis.

MATERIAL AND METHODS:

Chemicals and Reagents: n-butanol, Ethanol 96%, Folin-Ciocalteu's reagent, Gallic acid, Magnesium sulfate, Sodium hydroxide, Quercetin. Methanol 99%, Sodium oxalate, Dragendorff's reagent, Ethyl acetate, Hydrochloric Acid 35%, Aluminum chloride, Sodium carbonate, Sodium chloride, Petroleum ether 40-60°C, Dichloromethane, Calcium chloride dihydrate, ferric chloride, n-Hexane, Sodium citrate.

Plant Material: The leaves were collected from Charef, Djelfa, Algeria which is an area characterized by a dry and cold semi-arid climate and located between longitude 2 ° 48 ′ 4 ″ East and latitude 34 ° 37 ′ 5 ″ North, and located about 50 km from town of Djelfa. The identification was performed by a botanist in the Faculty of Science of Nature and Life at the University of Djelfa, Algeria, after this procedure, the leaves were air-dried and then powdered and stored in an opaque glass bottle.

Phytochemical Screening: Phytochemical screening was performed using the method cited by M. Barbouchi [24]. The experiments were based on the visual observation of a change in color or the formation of a precipitate after the addition of specific reagents [25].

Preparation of the Plant Extract:

Extraction of Polyphenols: Plant extracts were prepared consistent with a typical protocol with a slight modification. The powdered leaves (7g) were extracted by using a Soxhlet system with 150 ml of polarity solvents increased (petroleum ether followed by dichloromethane, ethyl acetate, n-butanol, and ended by water distilled) [26]. The extracts were filtered using Whatman paper and concentrated under Vacuum with Rotary Evaporator. The presence of polyphenols and flavonoids in the extracts fractions was confirmed by the ferric chloride and the cyanidin reaction

respectively [25]. These extracts were stored in a refrigerator at 4°C until use.

Extraction of Alkaloids: The extraction method of alkaloids is described in the section determination of the total content of alkaloids.

Determination of Total Phenolic Content (TPC): The samples were prepared by dissolving the organic extracts obtained during the extraction in 60 ml of ethanol and the aqueous extract in 60 ml of distilled water. Folin- Ciocalteu assay method was used for the determination of the total polyphenolics content with a few modifications [26]. The reaction mixture was prepared by mixing 0.1 ml of the different extracts, 0.4 ml of distilled water, and 0.25 ml Folin-Ciocalteu's reagent was added to the mixture and shaken well for 2 minutes. After that 1.25 ml of Na_2CO_3 20 %was added to the mixture. After incubation for 40 min at room temperature, the absorbance of the mixture was read at 750 nm by ultraviolet-visible spectrophotometer (BECKMAN DU 520UV-VIS). When making the blank preparation, the extract is replaced with 0.1 ml of distilled water. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard curve of gallic acid (20 to 200 µg/ml). Total polyphenolics were expressed as the microgram gallic acid equivalent (µg GAE)/g) of dry weight.

Determination of Total Flavonoids Content (TFC): The total flavonoid content was measured and quantified according to the Aluminum chloride method [27]. 1ml of each extract was added to 1 ml of $AlCl_3$ 2% solution. After incubation at room temperature for 10 minutes, the absorbance of the mixture was read at 430 nm. A calibration curve was made with quercetin (0-100 µg/ml). The total flavonoid content was expressed as the microgram quercetin equivalent per gram dry weight of the plant material (µg QE/ g). All samples were analyzed in three replicates.

Determination of Total Content of Alkaloids: The alkaloid-rich fraction prepared according to general procedure with some modifications [1, 28]. 8 g of the plant material was defatted with n-hexane and after filtration, the dried powder (7g) was extracted with 50ml MeOH for 2 h with magnetic agitation at room temperature, the operation was repeated twice, and the resulting MeOH extract was filtered and concentrated, the latter was dissolved in water, acidified with 2M HCl solution to pH 2, and extracted with $CHCl_3$. The aqueous layer was then basified with NaOH to pH 11and extracted with $CHCl_3$. The $CHCl_3$ extracts were dried with Mg_2SO_4 and concentrated to obtain the alkaloid-rich fraction (0,1012g). Phytochemical confirmation was carried out using Dragendorff's reagent.

***In-vitro* Antilithiatic Activity:** The 100% concentration of polyphenols and flavonoids fractions inhibitor is prepared by dissolving the organic extract in 60 ml of ethanol and aqueous extract in 60 ml of water distilled from this inhibitor; several concentrations are prepared using NaCl 0,15M as solvent. After several tests of the inhibitors, we have only chosen the concentrations which give reliable results (10%, 25%, 50%, and 100%). DMF: dichloromethane fraction (fraction rich in flavonoids), AF: aqueous fraction (fraction rich in polyphenols). The 100% concentration of alkaloid fraction inhibitor is prepared by taking 0,1012g of the dry residue with 60 ml of ethanol. From this inhibitor, we have prepared many dilute inhibitor solutions using the solvent sodium chloride (0.15 M). AlF: alkaloid fraction (10%, 25%, 50%, and 100%)

Inhibition of Calcium Oxalate Crystallization by Turbidimetric Method: The study of the crystallization of calcium oxalate is based on the research works illustrated by Chaudhary M. K. *et al* 29 and Eloumarie F. E. *et al* [30] with some modifications. The antiurolithiasis activity of alkaloids, polyphenols and flavonoids fractions was tested *in-vitro* by its inhibitory effect against calcium oxalate crystallization, this inhibition was examined in a mix containing calcium chloride dihydrate (7.5 mmol/l) and sodium oxalate (2.5 mmol/l), and these solutions were prepared using sodium chloride 0.15 M as a solvent. A series of inhibitors were prepared from the extracts as described above.

Study without and with Inhibitor: The formation of calcium oxalate crystals star when 7.5 ml of a sodium oxalate solution is added to 7.5 ml of a calcium chloride solution at 37 °C under magnetic

stirring. The absorbance of the mix was checked at 620 nm after 30min using a UV-visible spectrophotometer (Shimadzu 1240). The blank contains only the solution of calcium chloride dihydrate. Under the same operating conditions, a volume of 5 ml of sodium oxalate solution is added to a mixture containing 5 ml of calcium chloride solution and 5 ml of an inhibitor fraction at a well-defined concentration, after 30 min the absorbance of the mix was checked.

The effect of the inhibitory was compared to that of that of a 3 mM aqueous Sodium citrate trihydrate solution (positive control). All samples were tested in three replicates. The percentage inhibition I (%) produced by each concentration of inhibitor was calculated as follows:

$$\% \text{ inhibition} = (AbsC - AbsT) / AbsC$$

Where, Abs_C: Absorbance Control (Absorbance without inhibitor). Abs_T: Absorbance Test (Absorbance in the presence of inhibitor).

Dissolution of Calcium Oxalate by Gravimetric Method: The study of the efficacy of these fractions on the dissolution of calcium oxalate crystals were tested using the process illustrated by A. Bensatal [31], M Rossi [32], and R. Kachkoul [33].

Preparation of a Precipitate of Calcium Oxalate: In centrifuge tubes, we pour a volume of 2 ml of sodium oxalate 2.5 mmol / l (pH 7) on the same volume of solution calcium chloride (pH 6), leave the mixture under a temperature of 37°C for 30 minutes for the formation of a precipitate of calcium oxalate.

The tubes were then centrifuged at 6000 rpm using a SIGMA 2-16P centrifuge for 16 min, the supernatant was removed. Then the precipitates were washed with distilled water and again centrifuged as mentioned above. After these procedures, the supernatant was removed and the tubes were oven-dried at 70°C until a constant mass was obtained, and then reweighed to measure the mass of the precipitate.

Ability of Fractions to Dissolve the Calcium Oxalate Precipitate: In the centrifuge tubes, a volume of 4 ml of each fraction at various concentrations was poured on the calcium oxalate precipitate, and the tubes were incubated at 37 °C for 30 minutes.

The tubes were centrifuged and the precipitate was cleaned, dried, and weighed as mentioned above. The effect of dissolving was compared to that of a 3 mM aqueous Sodium citrate trihydrate solution. Three replicas were taken for each experiment. The ability to dissolve the precipitate was determined using the following formula:

$$A (\%) = ((W_{initial} - W_{final}) / W_{initial}$$

Where, A (%): the percentage of the ability to dissolve the precipitate. $w_{initial}$: the weight of the precipitate in the absence of fraction. w_{final}: the weight of the precipitate in the presence of fraction (after the incubation).

Statistical Analysis: The data were recorded as means ± standard deviation of three measurements. Difference among data was statistically analyzed using One-way ANOVA followed by the Tukey multiple comparison test to determine the level of significance using the software Graph Pad Prism version 8.0.2. A statistical difference of $P < 0.05$ was considered significant in all cases.

RESULTS:

Phytochemical Screening: A preliminary phytochemical analysis shows the existence of different secondary metabolites and the results are shown in the **Table 1.**

TABLE1: PHYTOCHEMICAL SCREENING OF *ZIZIPHUS LOTUS* EXTRACTS FRACTION

Extracts	Yield (%)	Polyphenols	Flavonoids
PEF	3.25	+	+
DMF	4.01	+	+
EAF	3.45	+	+
BF	9.61	+	+
AF	12.23	+	+

PEF: Petroleum ether fraction, DMF: Dichloromethane fraction, EAF: Ethylacetate fraction, BF : n Butanol fraction, AF: Aqueous fraction.

Quantitative Estimation of Secondary Metabolites: The total polyphenol compounds, and flavonoids, were quantified in the fractions: Petroleum ether, Dichloromethane, Ethyl acetate, Butanol, and Aqueous, the outcomes were displayed in **Table 2.**

TABLE 2: CONTENTS OF POLYPHENOLS AND FLAVONOIDS IN EXTRACTS FRACTIONS

Extracts	Total polyphenols (µgGAE)/g)	Total Flavonoids (µg QE /g)
PEF	1198,696 ± 99,848 [c]	97,340 ± 8,437[c]
DMF	3354,861 ± 343,163[c]	2446,064 ±63,606 [b]
EAF	3164,536 ±206,120 [c]	591,453 ± 17,9483[b]
BF	14985,212 ± 571,139[b]	1875,037 ± 138,941
AF	15270,751 ± 290,419[b]	1745,864 ± 8,690[a]

Data are reported as mean (n=3) ± SD (standard deviation). Values (a to c) that do not share a common letter in the same column are significantly different ($p < 0.05$). a represents the minimum value, c represents the maximum value.

The total alkaloid fraction (AlF) is 1.26%, and the results of the quantification of the polyphenols and flavonoids are calculated relative to the calibration curves previously defined (correlation of polyphenols: $Y = 0.00794 X - 0.00551$, $R^2 = 0,975$, correlation of flavonoids: $Y = 0.06569 X + 0.03804$, $R^2 = 0,993$). These results are shown in **Fig. 1** below.

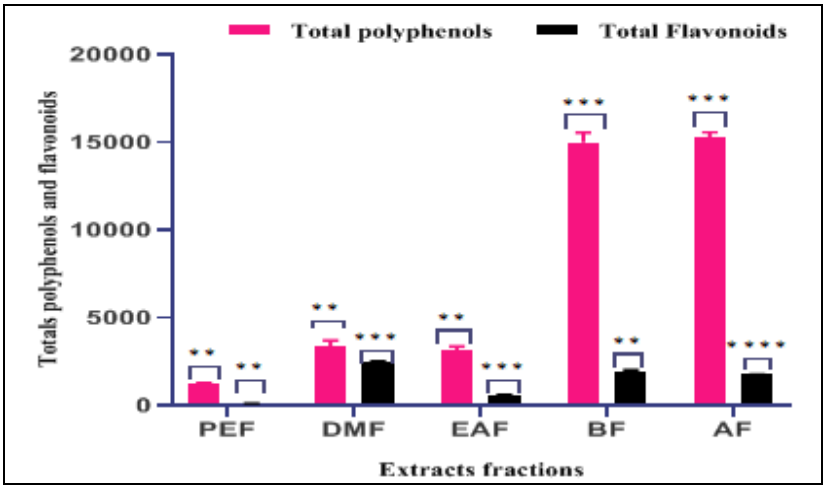

FIG. 1: TOTALS POLYPHENOLS AND FLAVONOIDS IN EXTRACS FRACTIONS. Values are expressed as mean ± standard deviation (n=3). **p<0.005, *** p<0.0001.

The results of the polyphenol assay indicate that the aqueous extracts are the richest in total polyphenols (15270.751 ± 290.419 µgGAE/g), conversely, the dichloromethane fraction is the richest in flavonoids (2446.064 ± 63.606 µg QE/g). The findings of the assay also indicate that the petroleum ether extract contains minimal quantities of flavonoids (97,340 ± 8,437) and polyphenols (1198, 696 ± 99,848).

Inhibition of the Calcium Oxalate Crystallization: The results of the inhibition activity of *Z. lotus* extracts on the crystallization of calcium oxalate are shown in **Table 3**.

The aqueous fraction had a more marked effect than the other fractions since, at a concentration of 100%, it caused an inhibition of 89.80 ± 0.69, while the other fractions (DMF) and (AlF) respectively caused inhibition of 84.17 ± 1.11; 78.90 ± 1.40 and 78.90 ±1.40, on the other hand, the citrate solution caused a minimum inhibition equal to 66.26±1,94.

The percentage of inhibition of the formation of calcium oxalate crystals as a function of different concentrations of extracts of leaves of *Z. lotus* (AF, DMF, AlF) and sodium citrate are shown in **Fig. 2**.

We note that the inhibiting activity of the crystallization of CaOx increases with increasing concentrations of the fractions in a dose-dependent manner from 10% to 100%, as shown in **Fig. 2.** The Sodium citrate at various concentrations was used as a positive control.

TABLE 3: VARIATION OF PERCENTAGE INHIBITION, IN TERMS OF TO THE FRACTIONS AT DIFFERENT CONCENTRATIONS

	C I (%)	10	25	50	100
Aqueous fraction(AF)	I (%)	38.72 ±3.01^{c}	60.75 ± 2.04^{b}	74.85±1.61^{b}	89.80 ±0.69^{a}
	Cv (%)	7.78	3.36	2.15	0.77
Dichloromethane fraction(DMF)	I (%)	42.59± 1.26^{b}	51,12 ±4,14^{c}	76.40± 0.92^{a}	84.17 ±1.11^{a}
	Cv (%)	2.97	8.95	1.21	1.32
Alkaloids fraction(AlF)	I (%)	32.10 ± 2.39^{c}	47.84 ± 4.71^{c}	58.27±5.35^{c}	78.90 ±1.40^{b}
	Cv (%)	7.47	9.85	9.18	1.77
Citrate (Control)	I (%)	26,13 ±2,48^{c}	45,73±2,58^{c}	61.65±3.41^{c}	66.26±1,94^{b}
	Cv (%)	9.51	5.65	5.53	2.93

CI (%) concentration of inhibitor, Cv (%) coefficient of variation, I (%) percentage of inhibition.

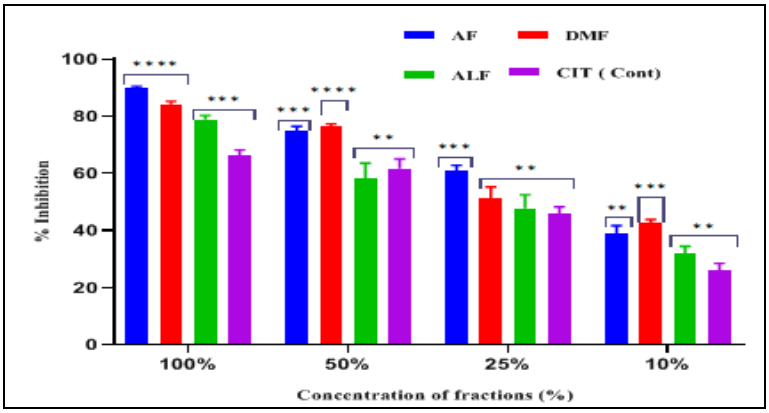

FIG. 2: EFFECT OF THE EXTRACTS FRACTIONS ON THE FORMATIO OF CALCIUM OXALATE. Values are expressed as mean ± standard deviation (n=3). **p<0.005, *** p<0.0005, ****p<0.0001.

Dissolution of Calcium Oxalate by Gravimetric Method: The dissolving capacity of calcium oxalate by the fractions of the extracts at different concentrations is shown in **Table 4.** The results are compared against sodium citrate.

TABLE 4: VARIATION OF PERCENTAGE DISSOLUTION, IN TERMS OF TO THE FRACTIONS AT DIFFERENT CONCENTRATIONS

	C I (%)	10	25	50	100
Aqueous fraction (AF)	A (%)	46,90±3,17^{c}	63,39±4,26^{c}	73,66±3,17^{b}	86,66±1,43^{a}
	Cv (%)	6,76	6,72	4,31	1,65
Dichloromethane fraction (DMF)	A (%)	26,32±2,23^{c}	58,71±2,74^{b}	69,92±3,15^{b}	80,85±2,03^{b}
	Cv (%)	8,46	4,67	4,51	2,51
Alkaloids fraction (AlF)	A (%)	6,15±0,19^{b}	16,06±1,06^{c}	32,71±2,55^{c}	66,81±4,13^{c}
	Cv (%)	3,10	6,62	7,80	6,18
Citrate (Control)	A (%)	21,20±1,72^{c}	51,10±4,61^{c}	66,84±3,22^{b}	75,38±2,03^{b}
	Cv (%)	8,12	9,03	4,82	2,70

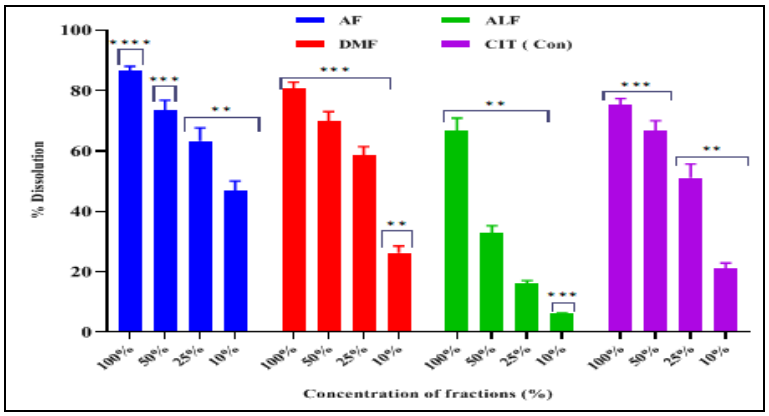

FIG. 2: EFFECT OF THE EXTRACTS FRACTIONS ON THE DISSOLUTION OF CALCIUM OXALATE. Values are expressed as mean ± standard deviation (n=3). **p<0.005, *** p<0.0005, ****p<0.0001.

Fig. 3 Presents the percent of calcium oxalate crystals dissolution by different concentrations of fractions aqueous, Dichloromethane, alkaloids, and Sodium citrate. The results show an increasing evolution of the capacity to dissolve the precipitate of calcium oxalate according to the increase in the concentration of the fractions, this change seems more important for the aqueous and dichloromethane fractions compared to the control solution (sodium citrate). On the other hand, the alkaloid fraction represents a less important dissolving capacity.

DISCUSSION: Urolithiasis is among the most common disorders in the world Where it is affected around 12% of the world's population by different forms of kidney stones, the recurrence rate in women is 47 to 60% and in men 70 to 80% [34]. The formation of kidney stones is a complex process and hyperoxaluria is one of the major risk factors for the renal stone composed of calcium oxalate (CaOx) crystals [35]. The values of the yields obtained vary from 1.26% to 12.23%, other studies give different results, this is due to climatic conditions and extraction techniques [15]. The extracts obtained show the presence of alkaloids, polyphenols and flavonoids during a qualitative preliminary phytochemical screening; and the results were also supported by previous studies [4, 6, 10].

In the present study, we chose a wide range (10% to 100%) of concentrations to evaluate it is inhibitory and dissolution against the formation of Calcium oxalate to have better comparison and to explore the optimal potential of the fractions, moreover, the techniques used do not contain any toxicity problem because the study was not conducted on the organism [36]. The Plant extracts contain several secondary and primary metabolites, but their respective therapeutic effects are not well defined; for this reason, it is necessary to separate and identify their active ingredients and to assess these therapeutic effects *in-vitro* and then *in-vivo*. First, we measured the contents of polyphenols and flavonoids in extracts of *Z. lotus* leaves (petroleum ether, dichloromethane, ethyl acetate, n butanol, and aqueous extract). On the other hand, a quantification of the total alkaloids was carried out by the gravimetric method. We noted that the extracts had a variable content of polyphenols and flavonoids. Secondly, to study the antilithiasis activity we have chosen the extract of dichloromethane which is rich in flavonoids (2446, 064 ± 63, 606) and the aqueous extract which is rich in polyphenols (15270, 751 ± 290, 419) and also the fraction of alkaloids (1.26%), this choice was based on recent studies that have shown that some metabolites such as saponins, flavonoids, polyphenols, and polysaccharides contribute significantly to the antiurolithiatic activity [30, 37-39].

The increase in the concentration of each fraction indicates that the formation of calcium oxalate particles has decreased. On the other hand, the dissolving activity is calculated after the lithiasis formation. The use of different concentrations of the polyphenol fraction resulted in a very significant reduction ((a****) and (b***)) in inhibition, but a small significant effect (c**) was observed for a lower dose at (10%). According to study [19], the main chemical constituents present in antiurolithic medicinal plants are respectively flavonoids (80%), alkaloids (65%), saponins (57.5%), tannins (47.5%), and compounds phenolic (42.5%). The comparison of the effects of inhibition of crystallization for the three fractions shows that the polyphenolic extract is more efficient than the two fractions which is even more efficient than the positive control, this result can be explained by the presence of polar compounds in the polyphenols fraction [25].

The–oxygen and nitrogen atoms present in polyphenols fraction (Resveratrol and Pyrogallol), flavonoids fraction (Quercetin, kaempferol), and alkaloids fraction (Lotusanine A, B, Sanjoinenine, Sanjoinine F, and Frangufoline) respectively can complex free Ca^{2+} in solution, increase Ca^{2+} enrichment and form high-energy interface on of these metabolites surface, so the chelating ability of Ca^{2+} was enhanced depending on the number of these atoms. Consequently, the calcium ion amount bound to the oxalate ion (Ox^{2-}) decreased which inhibited the formation of calcium oxalate stones [39, 40]. According to the study of Saha S *et al* [41] the effect of phenolic compounds on the formation of urolithiasis of calcium oxalate type could result not only from the 'electrostatic interaction between polyphenols and calcium ions but also from the interaction of type hydrogen between polyphenolic compounds and oxalate ions.

The study of the polyphenolic content of the leaves of *Z. lotus* grows in Algeria revealed the existence of flavonoids [7] of which catechin, is known for their antilithiasic effect [42]. For the dissolution the adsorbed inhibitor exerts an interfacial pressure on the interactions of the crystal network between the oxygen and nitrogen atoms of the inhibitor and the carboxylic acid of the surface oxalates (Ox) formed *via* a calcium bridge, (inhibitor) $O^{-}_{(inhibitor}$ Ca^{2+} $^{-}OOC_{(Ox, COD)}$ or $N^{-}_{(inhibitor)}$ Ca^{2+} $^{-}OOC_{(Ox, COD)}$ [43]. The chelation sites in forming the polyphenols and flavonoids complexes are illustrated in **Fig. 4.**

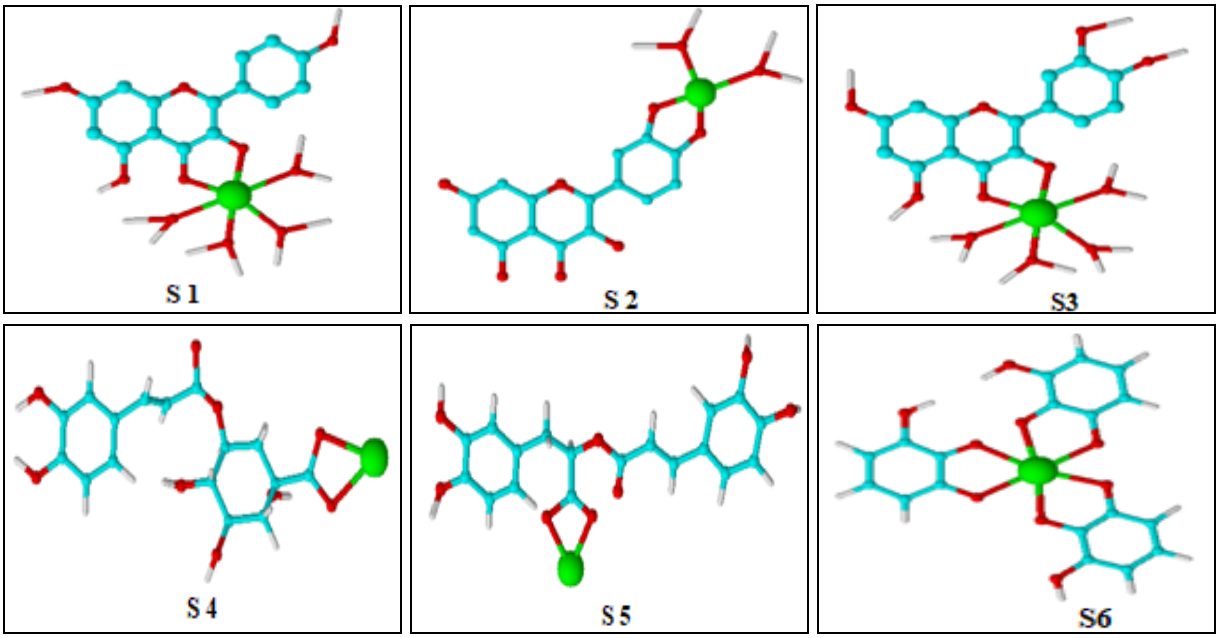

FIG. 4: CHELATION SITES IN FORMING THE POLYPHENOLS AND FLAVONOIDS COMPLEXES. S1. Proposed structures of kaempferol– Ca (II) complexes inspired by Jing *et al* [44]. S2. Proposed structure of quercetin-Ca (II) complexes [45]. S3. Proposed structure of quercetin-Ca (II) complexes inspired by Jing *et al* [44]. S4. Proposed structure of chlorogenic acid -Ca (II) complexes [46]. S5. Proposed structure of rosmarinc acid -Ca (II) complexes 46. S6. Proposed structure of Pyrogallol-Ca (II) complexes inspired by B Zhang *et al* [47].

CONCLUSION: In this study, we examined the efficacy of three fractions of secondary metabolites isolated from the leaves of .*Z lotus* extracts on the inhibition and dissolution of calcium oxalate stones. The results of this study allowed us to classify the inhibitory power of the different fractions as follows: Aqueous fraction〉 Dichloromethane fraction 〉 Alkaloid fraction〉 Control.

On the other hand, the alkaloid fraction has a less weak capacity to dissolve the precipitate of calcium oxalate than the other fractions, even compared to the control solution. This activity may be due to the presence of quantified phenolic compounds in the fractions, for this reason, the plant is useful as a preventive and therapeutic natural substance for urolithiasis. Further studies are needed to identify the compounds responsible for this activity and, in addition, further work in vivo is needed to better understand the mechanism and therapeutic effect of these fractions.

ACKNOWLEDGEMENTS: The author is thankful to the laboratory members of chemistry. Faculty of Exact Science and Computer Science, University Djelfa, Algeria

CONFLICTS OF INTEREST: The author has no conflicts of interest.

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How to cite this article:
Bensatal A: *In-vitro* inhibition and dissolution of calcium oxalate stones by the fractions of the *Ziziphus lotus* L. leaves. Int J Pharm Sci & Res 2024; 15(3): 844-53. doi: 10.13040/IJPSR.0975-8232.15(3).844-53.