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ORIGINAL ARTICLE

Food Chemistry, Engineering, Processing and Packaging

Phenol content, potential antioxidant and anti-urolithic effects of fruit and leaf extracts from Algerian *Capparis spinosa* L.

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ABSTRACT

Background: Ethnobotanical studies have historically documented the traditional medicinal use of Caper (*Capparis spinosa* L.) in treating kidney stones and other chronic renal ailments. **Aims:** This study aimed to evaluate the phenolic content, antioxidant capacity, and potential inhibitory effects of *Capparis spinosa* L. fruit and leaf extracts on calcium oxalate nucleation and aggregation. **Material and Methods:** Plant material was collected from the Tessala Mountains in Algeria. Total phenolic content was determined using the Folin-Cioclateu method, while *in vitro* antioxidant activity was assessed via the DPPH assay. *In vitro* anti-urolithic testing involved the use of turbidimetry in kinetic experiments to evaluate calcium oxalate nucleation and aggregation. **Results:** The IC_{50} values for antioxidant activity against DPPH were 8.67 mg/mL for fruit extracts and 0.57 mg/mL for leaf extracts. Furthermore, the investigated extracts exhibited a concentration-dependent inhibition of calcium oxalate crystal nucleation and aggregation. At the highest concentrations of leaf and fruit extracts, the corresponding inhibition rates were 74.47% and 66.06% for the nucleation test and 67.75% and 54.19% for the aggregation assay, respectively. **Conclusions:** These findings substantiate the traditional usage of *Capparis spinosa* L. in managing urinary lithiasis, suggesting its potential application as either a preventive measure or a therapeutic intervention for urinary stone formation.

Keywords: Antioxidant, Calcium oxalate, *Capparis spinosa* L., Urolithiasis, Polyphenols.

ARTICLE INFORMATION

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1 Introduction

Urolithiasis, commonly known as kidney stones, represents a prevalent and widespread health concern impacting approximately 15% of the global population, as indicated by numerous research endeavors. Its incidence demonstrates a continual rise across both developed and developing nations [1, 2]. Urolithiasis denotes the crystallization of solid mineral and acidic salts within the urinary tract. Calculi can develop within diverse segments of the urinary tract, encompassing the renal parenchyma, ureters, and urinary bladder. Predominant varieties of kidney stones comprise calcium oxalate, calcium phosphate, uric acid, and magnesium ammonium sulphate [3, 4].

In Algeria, the incidence of urinary lithiasis exhibits a notably higher incidence in the southern region compared to other geographical areas of the country [5].

A predominant proportion of cases approximately 80% manifests with calcium oxalate-based stones, concurrently present the highest recurrence rate [6]. Several risk factors contribute to lithiasis, with several attributing dietary modifications. However, genetic predispositions,

physiological constitution, metabolic dynamics, medication utilization, infectious susceptibilities, sex disparities, age-related variables, and lifestyle determinants are also implicated [7].

Surgical intervention remains the primary recourse, particularly for symptomatic calculi; however, pharmaceutical therapy and dietary and hygienic recommendations remain employed to stop recurrence [8]. While pharmaceutical treatment entails several adverse effects and a notable recurrence, rendering the disease recurrent, surgical procedures are marked by high costs and invasiveness.

The utilization of medicinal plants for urolithiasis treatment is a longstanding practice in traditional medicine. Various plants, including *Phyllanthus niruri*, Couch grass, Chanca Piedra, Dandelion root, Nettle leaf, and Corn silk, renowned for their diuretic, antiseptic, and calculolytic attributes, have been employed for centuries. These botanical resources aid in dissolution and prevention of renal calculi formation, along with alleviation of associated symptoms such as pain and discomfort. Furthermore, their adoption presents a natural alternative to conventional medications, potentially circumventing adverse effects. [9-10].

In Algeria, several medicinal plants have been utilized in urinary afflictions management. These plants include *Eucalyptus globulus* L., *Paronychia argentea* L., *Punica granatum*, *Tamarix gallica* L., and *Palenis spinosa* L [12].

Capparis spinosa L., commonly known as Caper, is a perennial plant classified under the Capparaceae family. Beyond its culinary utility, *Capparis spinosa* L. is esteemed for its pharmacological attributes and traditional therapeutic applications. Traditionally, *Capparis spinosa* L. has been lauded for its diuretic, anti-inflammatory, and antioxidant virtues. Pharmacological investigations further indicate its potential therapeutic efficacy in conditions such as rheumatism, arthritis, and gastrointestinal disorders. Furthermore, its antiviral and antimicrobial properties against various pathogens underscore its candidacy as a natural substitute for synthetic antibiotics [12].

An ethnobotanical survey conducted in Morocco suggests that the local population uses it as a traditional treatment for urinary lithiasis [13], but this anti-urolithic efficacy remains unexplored. Thus, this study aims to evaluate the antioxidant potential and *in vitro* effect on calcium oxalate crystallization of *Capparis spinosa* L. leaf and fruit extracts.

2 Material and Methods

2.1 Plant material harvesting and conservation

The botanical specimens utilized in this study comprise the leaves and fruits of *Capparis spinosa* L. (Family Capparaceae) (Figure 1). These specimens were procured from the Tessala commune situated in the western Algerian wilaya of Sidi Bel Abbes (GPS coordinates: 35° 16′ 27.25″, 0° 46′ 32.89″, 984.6412876), where the plant occurs naturally. Leaf collection was conducted in June 2021 while fruit collection occurred in late October of the same year. Subsequently, the harvested materials were subjected to drying and pulverization into a fine powder to facilitate extraction processes.

Figure 1. *Capparis spinosa* L., Family Capparaceae (plant, leaf, and flower).

Dr Nazim Bellifa identified the plant and an herbarium was deposited at the COSNA Laboratory - Faculty of Science – Tlemcen University.

2.2 Extraction procedure

The extraction process entailed maceration for 72 h of 10 g of powdered material from both the leaves and the fruit which were placed in Erlenmeyer flasks containing 100 mL of a solvent mixture comprising methanol and water (80:20). Subsequently, the extracts were then filtered and stored at low temperatures in opaque vials until required for further analysis [14].

2.3 Quantification of phenolic compounds

The total polyphenol content of extracts was determined using the Folin-Ciocalteu method [14]. To achieve this, 30 µL of the 1/10th diluted hydro-methanolic extract was mixed with 2.5 mL of Folin-Cioclateu reagent (1/10). Following a 3-minute incubation period, the mixture was incubated with 2 mL of sodium carbonate solution (Na_2CO_3 7.5%). Subsequently, the prepared samples were incubated in darkness for 30 min, after which the absorbance was measured at 760 nm. The total phenolic content was expressed in micrograms of gallic acid equivalents per milligram of dried plant material (µg GAE / mg dry weight).

2.4 Determination of antioxidant activity

The antioxidant activity was assessed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging method [14]. Briefly, 100 µL of various concentrations of each extract were added to 3.9 mL of freshly prepared DPPH methanolic solution (0.025 g/L). After incubation for 30 minutes, the reduction in absorbance was determined at 515 nm. The radical scavenging activity was expressed as the inhibition percentage (%) of free radical by the sample and calculated accordingly:

$$\text{Scavenging effect (\% Inhibition)} = [(A(\text{control}) - A(\text{sample})/A(\text{control})] \times 100$$

Where A(control) represents the absorbance value of the control (DPPH solution without extract), and A(sample) represents the absorbance value of the sample (Extract).

The IC_{50} was calculated graphically by linear regressions of the plotted graphs.

2.5 *In vitro* assessment of antiurolithic effect

The potential anti-urolithic properties of extracts from *Capparis spinosa* L. leaf and fruit were evaluated using three distinct methods. These methods included the examination of calcium oxalate crystallization kinetics using turbidimetry, optical microscopic analysis of crystallization patterns, and an aggregation test.

2.5.1 Study of calcium oxalate crystallization kinetics by turbidimetry

The modified turbidimetric model, according to the protocol described by Hess et al., [15] was employed to investigate inhibitors of oxalocalcic crystallization. Solutions of calcium chloride and sodium oxalate were prepared at final concentrations of 5 mol/L and 7.5 mmol/L, respectively, in a buffer solution containing Tris HCl 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. All solutions were maintained at 37°C with continuous stirring [9].

The crystallization reaction involving the predicted crystal growth, is represented as:

$$CaCl + NaC_2O_4 \rightarrow 2CaCO_4 + 2NaCl$$

Experimental procedures were conducted as follows:

- *Negative control group (without extract):*

  1 mL of calcium chloride was mixed with 1 mL of distilled water in a blank reading vessel, followed by the addition of 1 mL of sodium oxalate solution to initiate crystallization.

- *Test groups:*

  1 mL of calcium chloride solution was mixed with 1 mL of extract at concentrations ranging from 1 mg/mL to 10 mg/mL in a quartz cuvette. Upon achieving stable absorbance, 1 mL of anhydrous sodium oxalate solution (NaC_2O_4 7.5 mM) was added to the cuvette, and measurements were promptly initiated.

- *Positive control group:*

1 mL of calcium chloride solution was mixed with 1 mL of a 6 mM citric acid solution.

Crystallization was triggered by the addition of 1 mL sodium oxalate solution. The optical density of the solution was measured at 620 nm for 10 min every 10 seconds using a UV/VIS spectrophotometer. The nucleation rate was estimated by comparing the induction time in the presence of the extract with that of the control. The percentage of inhibition in relation to growth is calculated using the formula:

$$\% I = (1 - Pai / Psi) \times 100$$

Where Pai represents the turbidimetric slope in the presence of plant extract, and Psi represents the turbidimetric slope in the absence of plant extract.

2.5.2 Optical microscope study of crystallization

We utilized a technique introduced by Kavanagh et al., [16] with slight adjustments as required. In this procedure, oxalate was introduced simultaneously to the negative control (1 mL $CaCl_2$ with 1 mL distilled water), positive control (1 mL $CaCl_2$ with 1 mL citric acid), and samples (1 mL $CaCl_2$ with 1 mL of the two extracts at varying concentrations). At the growth stage corresponding to t=2 min, a drop was placed between a slide and coverslip, and the crystals were observed under optical microscopy at G×10 and G×40 magnification [16].

2.5.3 Aggregation test

Calcium oxalate (CaOX) crystals were prepared *in vitro* as follows: solutions of calcium chloride (50 mM) and sodium oxalate (50 mM) were mixed and heated in a water bath for one hour at 60°C. The resulting solution was cooled overnight and filtered twice on Wattman paper the following day. The residue obtained was weighed and dried in an oven at 37°C. A CaOX solution of 1 mg/mL from the dry residue was prepared in Tris-HCl 0.05 mol/L and NaCl 0.15 mol/L buffer at pH 6.5. For each extract, from a stock solution of 100 mg/mL, the following concentrations were prepared: A1(1mg/mL), A2 (2 mg/mL), A3 (5 mg/mL), A4(7.5 mg/mL). Concurrently, two controls – positive (containing citric acid as an inhibitor) and negative (containing no inhibitor) – were prepared.

Each concentration (1 mL) was added to 1 mL of the pre-prepared CaOX solution. Absorbance was measured at 620

nm in the presence and absence of extracts every 30 min for a duration of 3 hours. The percentage inhibition of aggregation was calculated using the formula:

$$PA\ \% = [1-(Pi/Pc)] \times 100$$

Where PA represents the percentage of aggregation inhibition, Pi is the turbidimetric slope in the presence of extract, and Pc is the turbidimetric slope in the absence of extract [17].

2.6 Statistical analysis

All measurements were performed in triplicate, and the experimental data were reported as mean ± standard deviation (SD) of three parallel measurements. IC_{50} values were determined by linear regression analysis. Microsoft Excel and SPSS (version 15, USA) were used for statistical analysis. The statistical difference between the results was determined using One-way ANOVA with a significance threshold of $p < 0.05$.

3 Results

3.1 Estimation of total phenols

Analysis of total polyphenol content revealed that leaves of *Capparis spinosa* L. exhibited the highest value, at 57.265 ± 0.00 µg GAE/mg dry weight. Fruits on the other hand, possessed a content of 32.295 ± 0.77 µg GAE/mg dry weight.

3.2 Determination of antioxidant activity

The DPPH assay was employed to assess the antioxidant potential of the hydro-methanolic extract obtained from both leaves and fruits of *Capparis spinosa* L. was determined using the DPPH assay. The antioxidant activity of these extracts is expressed as the percentage of inhibition, which subsequently serves as the basis for calculating the IC_{50}. The obtained results demonstrate dose-dependent antioxidant activity for the tested extracts. The fruit extract exhibited the highest antioxidant activity, with an IC_{50} value of 8.67 mg/mL. In contrast, the leaf extract displayed a lower IC_{50} value of 0.57 mg/mL, indicating superior antioxidant capacity against DPPH radicals.

3.3 *In vitro* assessment of anti-urolithic effect

Kinetics of calcium oxalate crystallization

The results of the analysis showed for the leaf extract 23.72% inhibition at the lowest dose (1 mg/mL) and 74.47% inhibition at the highest dose (10 mg/mL). For the fruit extract, the inhibition rate was 14.95% for the lowest dose (1 mg/mL) and 60.06% for the highest dose (10 mg/mL). Inhibition of calcium oxalate formation in the presence of the extract was compared with positive control (citric acid), which gave an inhibition of 88.38%. These findings suggest the presence of nucleation-inhibiting agents within both extracts, with leaves exhibiting a superior inhibitory capacity against calcium oxalate formation compared to fruits.

Figures 2 and **3** show crystallization curves in the presence of leaf and fruit extracts, negative and positive controls, and the results of the turbidimetric parameters are shown in **Table 1**.

Table 1. Turbidimetric parameters of oxalocalcic crystallization

Parameters	[mg/mL] *	Slope (OD/min)	Ti (min)	% ± SD
Negative control	/	0.2782	0.2279	/
Citric acid 6mM		0.0323	0.0161	88.38 ± 0.0363
Leaf extract	1	0.2122	0.0558	23.72 ± 0.0421
	2	0.1732	0.1481	37.74 ± 0.0262
	5	0.1059	0.1461	61.93 ± 0.0347
	7.5	0.0874	0.1512	68.58 ± 0.0192
	10	0.071	0.1692	74.47 ± 0.0497
Fruit extract	1	0.2366	0.1326	14.95 ± 0.0140
	2	0.1351	0.1498	51.43 ± 0.0136
	5	0.1134	0.2464	59.23 ± 0.0257
	7.5	0.106	0.2493	61.89 ± 0.0351
	10	0.0944	0.2643	66.06 ± 0.0246

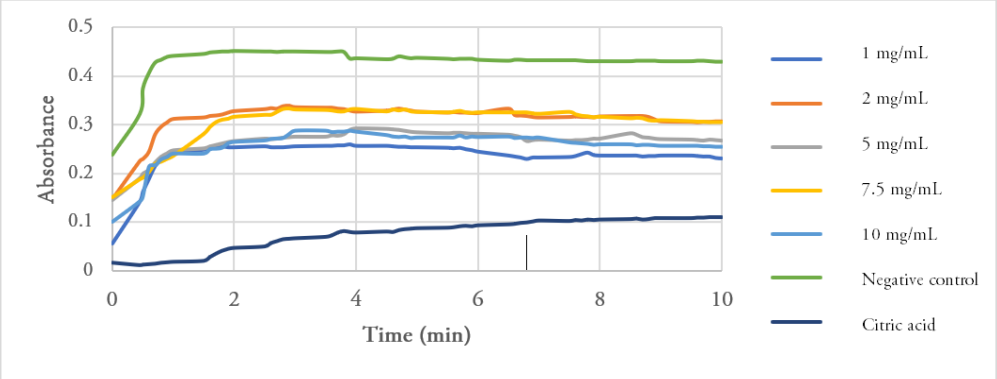

Figure 2. Crystallization curves in the presence of leaf extract, negative control, and positive control

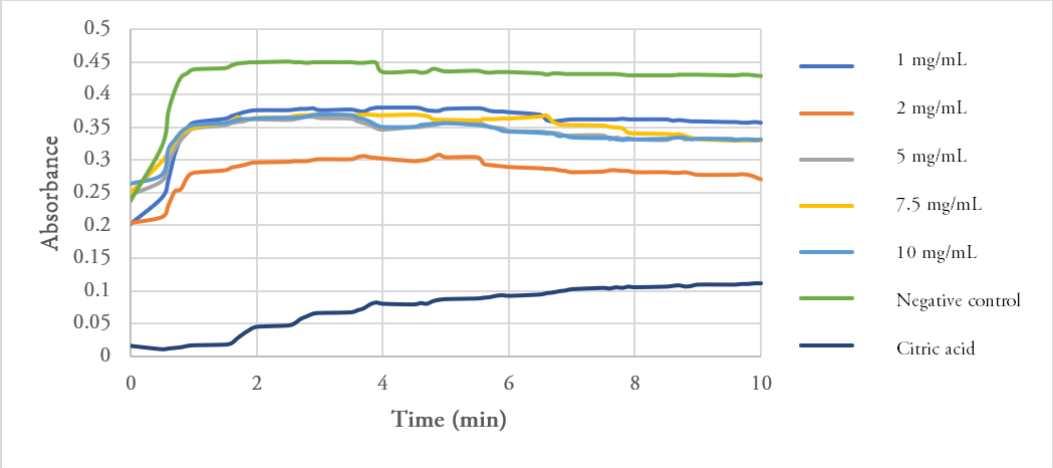

Figure 3. Crystallization curves in the presence of fruit extract, negative control, and positive control

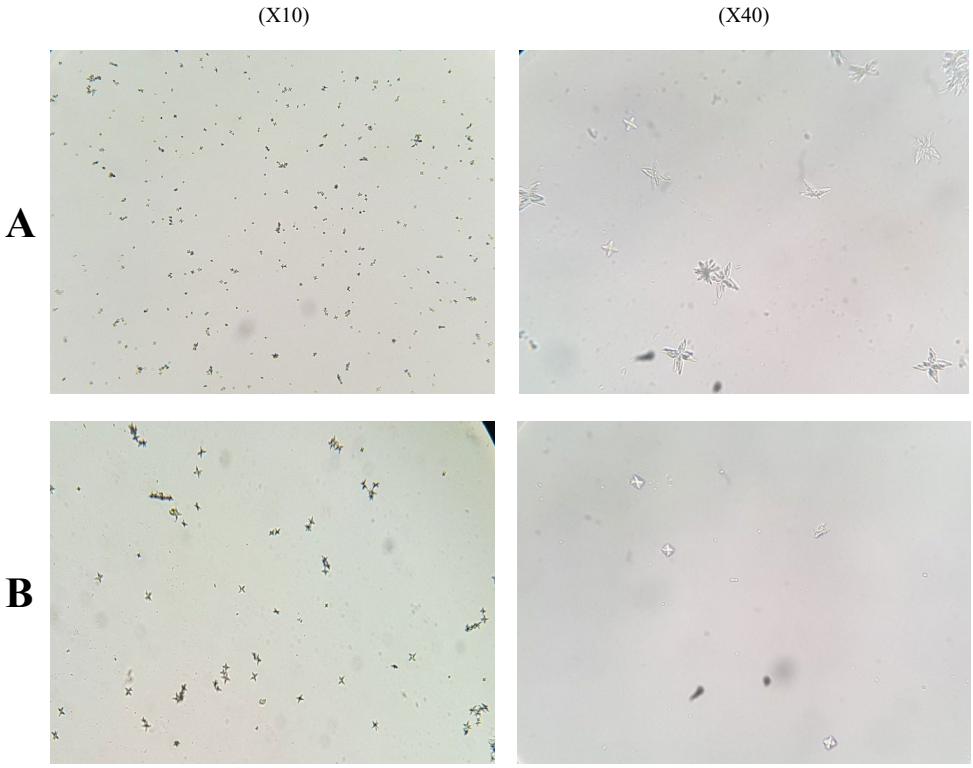

Figure 4. Microscopic observation of inhibition of oxalocalcic crystallization. A: without inhibitor, B: in the presence of citric acid (left G10x10, right G10x40)

Microscopic observation of oxalocalcic crystallization

The time of the photographs (t= 2 min) corresponds respectively to the growth stages for trials in the absence of any inhibitor (Figure 4, A), with citric acid (Figure 4, B), and in the presence of leaf and fruit extracts (Figure 5).

As evident from Figure 4. the number of crystals and oxalocalcic aggregates is significantly reduced when the positive control (citric acid) is present.

Furthermore, a comparison of photographs corresponding to the growth phase of calcium oxalate crystallization in the absence of extract (Figure 4) with those obtained in the presence of leaf, fruit extracts at low concentrations (1 mg/mL

to 2mg/mL) a negligible effect on crystal number and morphology. In contrast, inhibition increased with higher concentrations (5 mg/mL, 7.5 mg/mL, 10 mg/mL). Hydro-methanolic extracts of *C. spinosa* L. leaves and fruits inhibited crystal growth, although a remarkable reduction in the number of crystals and oxalocalcic aggregates was observed in the leaf extract compared to those formed with the fruit extract.

Several tests were carried out in the presence and absence of inhibitors (citric acid and the hydromethanolic extract of the leaves and fruit of *Capparis spinosa* L.) to determine the critical parameters for assessing aggregation, namely the turbidimetric slope and the linear regression coefficient (**Figures 6 - 9**).

The results obtained from the turbidimetric assay

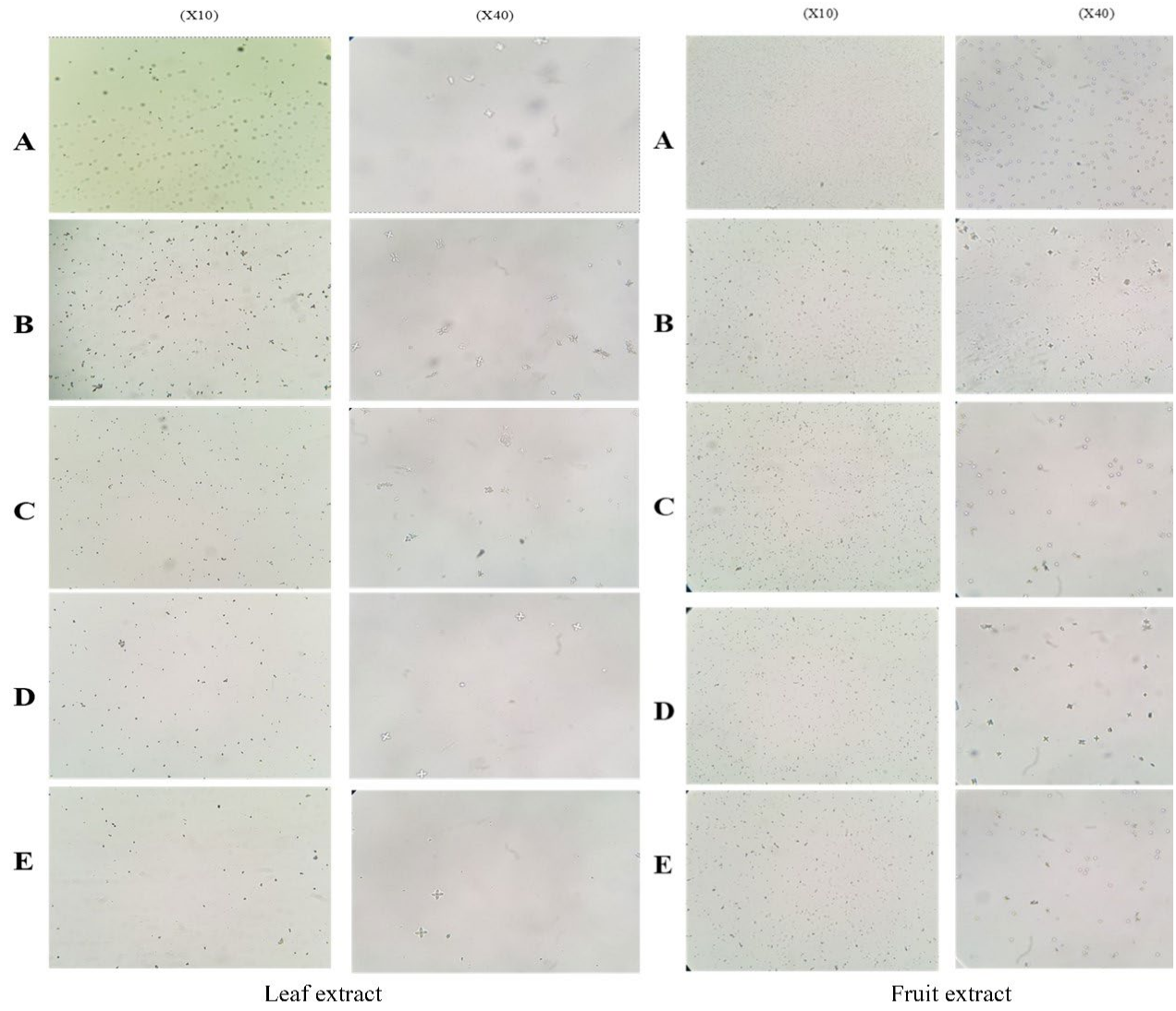

Figure 5. Microscopic observations of the inhibition of oxalocalcic crystallization by different concentrations of leaf and fruit extracts (A: 1 mg/mL, B: 2 mg/mL, C: 5 mg/mL, D: 7.5 mg/mL, E: 10 mg/mL)

Aggregation test

The inhibitory effect of Caper leaf and fruit extracts was evaluated using a turbidimetric model to track the kinetics of calcium oxalate crystal aggregation.

demonstrate a progressive decrease in absorbance over time across the various concentrations of both extracts. As a result, we observed that the value of the turbidimetric slope increased with increasing extract concentration. On the other hand, the percentage inhibition of aggregation also increases proportionally with increasing extract concentration.

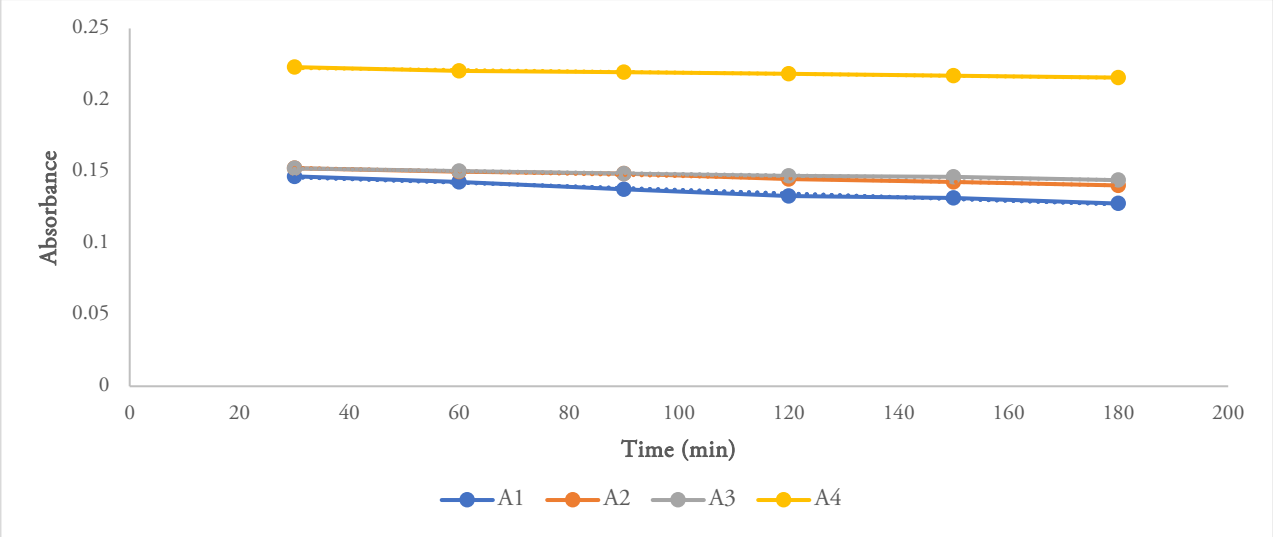

Figure 6. Aggregation kinetics in the presence of leaf extract from *Capparis spinosa* L. as a function of time.

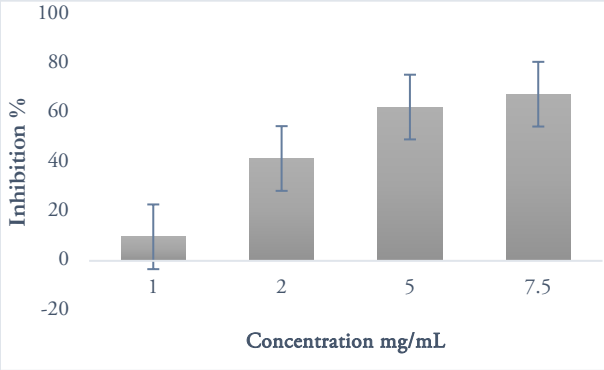

Figure 7. Inhibition (%) of different concentrations of *Capparis spinosa* L. leaf extract ($p = 0.023 < 0.05$)

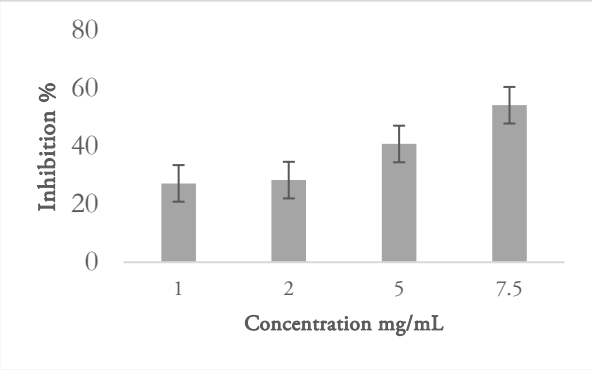

Figure 9. Inhibition (%) of different concentrations of *Capparis spinosa* L. fruit extract ($p = 0.013 < 0.05$)

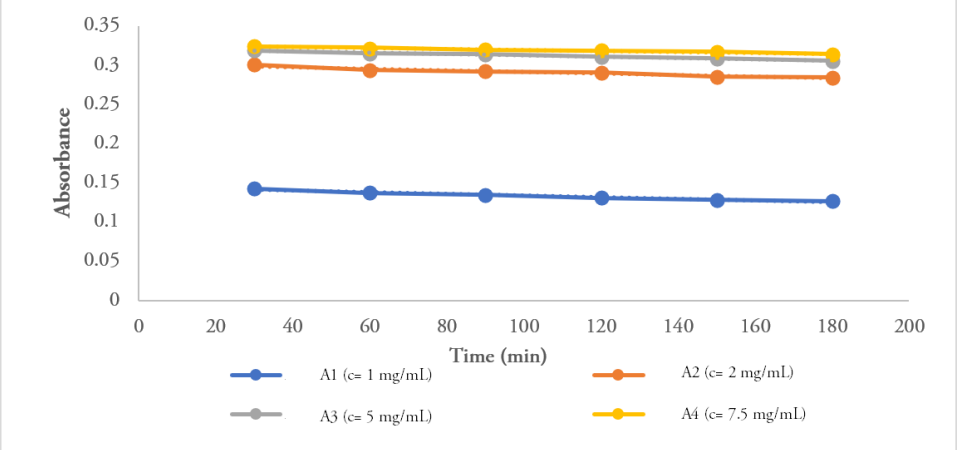

Figure 8. Evolution of aggregation kinetics in the presence of *Capparis spinosa* L. fruit extract as a function of time

4 Discussion

Analysis of total phenolics revealed fruit and leaf contents of 32.29 5 ± 0.77 and 57.265 ± 0.00 µg gallic acid equivalent /mg dry weight, respectively. These values differ from those reported in another study by Benzidane et al. [18], likely due to several factors such as the geographical location and harvesting time, extraction method, and solvent selection.

The DPPH assay demonstrated corresponding trends in antioxidant activity, with IC50 values of 8.67 mg/mL and 0.57 mg/mL for fruit and leaf extracts, respectively. The same result was found in a study carried out on different extracts of different parts of *Capparis spinosa* L., which showed that the methanolic extract of the leaves possessed a high antioxidant activity compared to the fruits [19].

The *in vitro* effect of *Capparis spinosa* L. extracts on urolithiasis was evaluated [20]. Numerous studies conducted in Algeria and Morocco highlighted the plant *Capparis spinosa* L. However, its anti-lithiasis properties remain unverified.

Our investigation focused on calcium oxalate nucleation, a critical step in kidney stone formation. The leaf extract displayed dose-dependent inhibition, ranging from 23.72% at 1 mg/mL to 74.47% at 10 mg/mL. The fruit extract also exhibited dose-dependent inhibition, albeit to a lesser extent (14.95% at 1 mg/mL to 60.06% at 10 mg/mL). Notably, the positive control, citric acid, achieved an inhibition rate of 88.38%. These findings suggest that both extracts possess anti-urolithic potential, with the leaf extract demonstrating a superior inhibitory effect on calcium oxalate crystal formation compared to the fruit extract.

Sbahi et al. (2013) investigated the *in vitro* inhibitory effects of methanolic extracts from *Juniperus oxycedrus*, *Marrubium vulgare*, and *Inula viscosa* on calcium oxalate crystallization using a turbidimetric model. At a concentration of 10 mg/mL and an incubation time of 15 minutes, these extracts exhibited inhibition rates of 53.1%, 56.86%, and 56.59%, respectively [21]. Djaroud et al. [22] showed that the anti-lithiasis activity of the aqueous extract of *Hordeum vulgare*, using the same method provides a significantly lower activity with an inhibition rate of only 6.2% for a concentration of 1 mg/mL extract. Beghalia et al. [23] found that the anti-lithiasis activity of *Atriplex halimus* leaf extract was 92.80% for 30 min. Additionally, Saha and Verma [17] found that hydroalcoholic extract of *Bergenia ciliata* exhibited an inhibition rate of 62% at a concentration of 10 mg/Ml.

These comparisons show that extracts of *Capparis spinosa* L. are highly inhibitory to oxalocalcic crystallization *in vitro*.

To confirm these results, the number and size of calcium oxalate crystals were examined using optical microscopy. Crystals were observed in the absence of an inhibitor, with citric acid, and in the presence of leaf and fruit extracts from *Capparis spinosa* L. The results obtained revealed a dose-dependent inhibition of crystal growth, consistent with the kinetic data obtained earlier. Notably, this inhibition occurred at the growth stage, aligning with the results from the oxalocalcic crystallization kinetics assay. A comparison with previous research by Sbahi [21], who reported a significant decrease in crystal aggregate size and number using methanolic extract of *Marrubium vulgare* at a concentration of 10 mg/mL at t = 5 min, *Inula viscosa* at a higher concentration of 30 mg/mL at t = 5 min and *Juniperus oxycedrus at* a concentration of 20 mg/mL at a longer time t = 15 min. This allows us to confirm the efficacy of hydromethanolic extracts of *Capparis spinosa* L *in vitro* in inhibiting oxalacalcic crystallization.

The aggregation test demonstrated a dose-dependent inhibitory effect of the leaf extract with a percentage inhibition ranging from 9.75% to 67.57% at the highest concentration. However, fruit extract exhibited a lower inhibitory effect, ranging from 27.21% to 54.19% across the tested concentrations. These findings confirm the superior anti-lithiasic potential of the leaf extract compared to the fruit extract. Compared with other *in vitro* studies carried out on different parts of different plants, we found that the study by Sasikala et al. [24] showed that the methanolic extract of the leaves and stems of *Rotula aquatica Lour* was inhibited by 10 to 40% for the leaf extract and 20 to 50% for the stem extract at concentrations ranging from 100 to 500 Ug/mL. These results were lower than those observed in our study. In contrast, the study carried out by Saha [17] showed that the hydromethanolic extract of *Bergenia ciliata* rhizomes exhibited a percentage inhibition of 58 to 97% at concentrations ranging from 1 to 10 mg/mL. Whereas according to Kachkoul et al. [25], the methanolic extract of *Ammi visnaga* seeds and *Punica granatum* fruit showed a percentage inhibition of 64.29 to 77.12% and 74.90 to 83.46% for *Ammi visnaga* seeds and *Punica granatum fruit* successively over a concentration range of 0.25 to 2 g/L. Meanwhile, the study on *Herniaria hirsuta* extract exhibited 70 – 90% inhibition at concentrations ranging from 0.0625 to 1 mg/mL [26].

The anti-urolithic power of plants is likely attributable to their content of bioactive molecules such as polyphenols and flavonoids. In support of this, a study by Zeng et al. [27] demonstrated the efficacy of plant flavonoids against urinary lithiasis through both *in vitro* and *in vivo* studies. The proposed mechanisms of action for these flavonoids involve complexation with calcium ions, thereby reducing the availability of free calcium for oxalate crystal formation. Additionally, they may modulate the synthesis and expression of factors within the body that promote or inhibit stone formation. The observed correlation between anti-urolithic

activity and antioxidant capacity, as exemplified by *Capparis spinosa* L. in this study, warrants further investigation. These findings suggest the potential of *Capparis spinosa* L. extracts as inhibitory agents for the prevention and/or treatment of urinary lithiasis.

5 Conclusion

Medicinal plants with anti-lithiasis properties may offer a promising avenue for improved management of renal lithiasis and potential therapeutic options. This study aimed to investigate the effect of the Caper's leaf and fruit extracts on oxalo-calcic crystallization.

The analysis revealed a higher concentration of total phenolics in the leaves (57.265 mg GAE/g) compared to the fruits (32.295 mg GAE/g). The turbidimetric assay demonstrated a significant dose-dependent inhibitory effect of both extracts on calcium oxalate crystallization. the leaf extract exhibited a maximum inhibition of 68.58% at 7.5 mg/mL, while the fruit extract displayed a maximum inhibition of 61.89% at the same concentration. Microscopic observations confirmed these findings, visually demonstrating the dose-dependent inhibition of crystal formation by both extracts. The present study provides evidence that *Capparis spinosa* L. leaf and fruit extracts possess anti-lithiasic properties *in vitro*. The observed inhibition of calcium oxalate crystallization may be attributable to the presence of bioactive compounds, particularly polyphenols, found in higher concentrations within the leaves. These findings represent an initial exploration of the potential therapeutic application of *Capparis spinosa* L. for managing urinary lithiasis. Future endeavors will focus on *in vivo* testing of the extracts to confirm their efficacy and safety within a living organism. Additionally, the isolation and identification of the active components responsible for the anti-urolithic activity are crucial steps in understanding the mechanisms of action and potentially developing novel therapeutic strategies.

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