# INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION CAUSING KIDNEY STONES IN VITRO BY AN EXTRACT OF *Bluméa balsamifera*

**Nguyen Pham Tuan[a*], Bang Hong Lam[c], Nguyen Pham Tu[a], Le Thao Nguyen[b], Tran Duc Tai[b]**

*[a]An Giang Biotechnology Center, An Giang, Vietnam*
*[b]Gachon University, Seongnam, Gyeonggi, South Korea*
*[c]An Giang University, An Giang, Vietnam*
*[*]Corresponding author: Email: ngphamtuan1983@gmail.com*

**Article history**
Received: April 4th, 2020
Received in revised form: September 28th, 2020 | Accepted: October 26th, 2020
Available online: February 5th, 2021

## Abstract

*The inhibitory effect of Bluméa balsamifera extract on the nucleation, growth, and aggregation phases of calcium oxalate formation has been studied. Plant samples were extracted by the maceration method with 80.00% ethanol. The results showed that the moisture content of Bluméa balsamifera extract was 82.83% and the yield was 5.79%. Bluméa balsamifera extract contains bioactive compounds including alkaloids, flavonoids, saponins, terpenoids, tannins, and phenol. Bluméa balsamifera extract has the ability to inhibit the nucleation of calcium oxalate with an $IC_{50}$ value of 4.25 mg/ml. Bluméa balsamifera extract has a significant inhibitory effect on the growth of calcium oxalate crystals with an $IC_{50}$ value of 2.99 mg/ml. Finally, Bluméa balsamifera extract is capable of inhibiting the aggregation of calcium oxalate with an $IC_{50}$ value of 2.56 mg/ml.*

**Keywords**: Aggregation; *Bluméa balsamifera*; Calcium oxalate; Growth; Nucleation.

DOI: http://dx.doi.org/10.37569/DalatUniversity.11.1.691(2021)

Article type: (peer-reviewed) Full-length research article

# 1. INTRODUCTION

Kidney stones are hard solid crystals formed in the kidney. The stones have five forms: calcium oxalate, calcium phosphate, or a mixture of both crystals (accounting for 85% of monolithic and mixed forms), struvite, uric acid, and, rarely, cysteine. Calcium oxalate crystals are the main component of more than 60% of kidney stones in humans. The crystals exist in two main forms: calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD). In the human body, kidney stones have two sizes: small (< 5 mm) and large (> 10 mm). The human body can pass the small stones without affecting health. Conversely, large stones can clog the urinary tract causing pain (Atodariya et al., 2013). In addition, medical therapy is effective in the treatment of calcium oxalate stones but has a 50% risk of recurrence and many side effects, such as damage to blood vessels in the kidneys and surrounding organs, etc. (Trần, 2016). According to traditional experience, the leaves of the compassion plant *Bluméa balsamifera* are capable of treating urinary diseases and kidney stones. Research by Charlimagne and Rizalinda (2017) indicates that *Bluméa balsamifera* extract has the ability to reduce the size of calcium oxalate crystals. From there, the subject: Inhibition of calcium oxalate crystallization causing kidney stones in vitro by an extract of *Bluméa balsamifera* to evaluate the effect of inhibiting the formation of calcium oxalate crystals causing kidney stones of medicinal plants, aiming to create products with medicinal properties for prevention and treatment of diseases.

# 2. MATERIALS AND METHODS

## 2.1. Materials

Leaves of *Bluméa balsamifera* were collected from a greenhouse at the An Giang Biotechnology Center. Chemicals and equipment, such as a freeze dryer, spectrophotometer, sodium oxalate, Tris HCl, etc., were used.

## 2.2. Methods

### *2.2.1. Sample preparation*

The plant material was cleaned with distilled water and damaged leaves were removed. The material was shade dried with a freeze drier and then ground into powder for extraction. The extracted powder (200 g) was soaked with 80% ethanol with the ratio 1 to 10 (w/v) at room temperature for 72 hours and then filtered through Whatman filter paper No. 1. After the mixture was evaporated at 50 $^{\circ}$C to remove solvent, the crude extract was dried with a freeze drier and stored at -20 $^{\circ}$C.

### *2.2.2. Phytochemical screening of Bluméa balsamifera extract*

The phytochemicals screening method was based on the method of Yadav et al. (2014) (Table 1).

**Table 1. Screening method for bioactive compounds in the leaves of *Bluméa balsamifera* extract**

| Bioactive compound | Methods | Phenomenon |
|---|---|---|
| Alkaloids (Mayer) | 1 ml extract + drops of Mayer's reagent | Brown precipitation |
| Flavonoids | 1 ml extract + 2 ml $Pb(OAc)_4$ 10% | Yellow precipitation |
| Saponins (Foam) | 3 ml extract + 6 ml $H_2O$ → Heat | Foam appearance |
| Steroids (Salkowski) | 1 ml extract + 2 ml $CHCl_3$ + 2 ml $H_2SO_{4\ concentrated}$ | Red brown appearance between two layers |
| Tannins and phenol (Braymer) | 0.5 ml extract + 10 ml $H_2O$ + 2-3 drops of $FeCl_3$ 0.1% | Blue precipitation |
| Terpenoids | 2 ml extract + 2 ml $(CH_3CO)_2O$ + 2-3 drops of $H_2SO_{4\ concentrated}$ | Dark red color appearance |

*2.2.3. Nucleation assay*

The nucleation phase was initiated by adding calcium chloride 4 mM to sodium oxalate 50 mM in the absence or presence of inhibitors (sodium citrate, extract) at different concentrations (0.0625, 0.1250, 0.2500, 0.5000, 1.0000, 2.0000, 4.0000, 6.0000, and 8.0000 mg/ml). Both solutions were prepared in a buffer containing TRIS 0.05M and NaCl 0.15M at pH = 6.5. For the assay, 950 µl of calcium chloride was mixed with various concentrations of the inhibitors or blank (100 µl), then 950 µl of sodium oxalate was added and shaken well (Phatak & Hendre, 2015). The inhibition was determined by measuring the absorbance of the mixture at 620 nm, and the percentage of inhibition was calculated as:

$$\%Inhibition = [(C-S)/C] \times 100\% \quad (1)$$

where C is the optical density (OD) of the sample without inhibitors, and S is the OD of the sample in the presence of inhibitors at different concentrations.

The standard used to compare the efficacy of inhibiting nucleation, growth, and aggregation of calcium oxalate crystallization is sodium citrate. Evaluation of its inhibitory effect is based on $IC_{50}$.

*2.2.4. Growth assay*

A system of 4.0 ml was prepared to evaluate the inhibition capacity of the extract. We added 1.0 ml each of 4.0 mM calcium chloride and sodium oxalate to 1.5 ml of the buffer containing TRIS 10.0 mM and NaCl 90.0 mM at pH = 7.2. 30 µl of calcium oxalate monohydrate, which were buffered in sodium acetate 50 mM at the final concentration of 1.5 mg/ml and pH = 5.7. If the *Bluméa balsamifera* L. extract inhibits the growth of calcium oxalate, the depletion of oxalate ions, detected by a spectrophotometer at a wavelength of 214.0 nm, will be decreased because the reaction between calcium chloride and sodium oxalate with the crystal seeds leads to deposition of CaOx on the crystal

surfaces. The reaction was monitored for 600 s, and the values were recorded every 60 s (Chaudhary et al., 2010).

$$\%\text{Inhibition activity} = [(C-S)/C] \times 100\% \quad (2)$$

where C is the slope of the trend line without inhibitors, and S is the slope of the trend line in the presence of inhibitors.

*2.2.5. Aggregation assay*

The CaOx crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mM. The solution was equilibrated in a water bath at 60 $^{\circ}$C for 1 hour, then cooled at 37 $^{\circ}$C overnight to stabilize the crystals (i.e., not growing or dissolving in the solvent). The crystals were harvested by centrifugation at 14,000 rpm and evaporation at 37 $^{\circ}$C. CaOx crystals were used at a final concentration of 1 mg/ml, buffered with TRIS 0.05 M and 0.15 M at pH = 6.5. The sample without any extract was used as a control to compare with various concentrations of inhibitors (extract and sodium citrate). The absorbance at 620 nm was recorded at 30, 60, 90, 180, and 360 minutes (Saha & Verma, 2013). The percentage of inhibition was calculated as:

$$\%\text{Inhibition} = (1-Si/Sc) \times 100\% \quad (3)$$

where Si is the slope of the trend line in the presence of the inhibitors, and Sc is the slope of the trend line without inhibitors.

$IC_{50}$ definition: $IC_{50}$ is the measure used to evaluate the strong or weak inhibitory capacity of the sample. $IC_{50}$ is defined as the concentration (mg/ml) of the sample that can inhibit 50% of free radicals, cells, or enzymes. The more active the sample, the lower the $IC_{50}$ value will be.

How to determine $IC_{50}$: An investigation of a sample's activity is conducted at many different concentrations. For samples that vary linearly with concentration, draw a line $y = ax + b$ through all points (where y is % inhibition and x is concentration). For samples that vary nonlinearly with concentration, choose two inhibitory concentrations above and below 50% and draw a straight line to get the equation $y = ax + b$ with known coefficients a and b. From the known $y = ax + b$ equation, replacing $y = 50\%$ in the equation will yield the value of x, which is the 50% free radical inhibitory concentration ($IC_{50}$).

## 2.3. Statistical analysis

Data collected from the experiment were processed with Microsoft Excel. Statistical analysis was conducted with Statgraphics Plus 16.0.

## 3. RESULTS AND DISCUSSION

### 3.1. The method of *Bluméa balsamifera* leaf

The extraction procedure was performed on leaves of *Bluméa balsamifera* weighing 515 grams (dry). Moisture and yield of *Bluméa balsamifera* extract were 82.83% and 5.79%, respectively (Table 2). Ethanol is the appropriate solvent for extraction. If water is used as the sole solvent for extraction, the sample has a high risk of contamination with organic acids, glucose, or protein, which are dissolved in the water, thereby causing a negative effect to the bioactive compound qualitative or quantitative process. Furthermore, if 96.00% ethanol is used as the sole solvent, the yield is decreased. Therefore, 80.00% ethanol was used as the effective solvent for extraction because it gives a high extraction productivity and removes all bacterial contamination (Bandar et al., 2013).

**Table 2. Extraction results of *Bluméa balsamifera***

| Evaluation | Results |
|---|---|
| Fresh weight (g) | 3,000.00 |
| Moisture content (%) | 82.83 |
| Dry weight (g) | 515.00 |
| Extract weight (g) | 29.83 |
| Yielding extraction (%) | 5.79 |

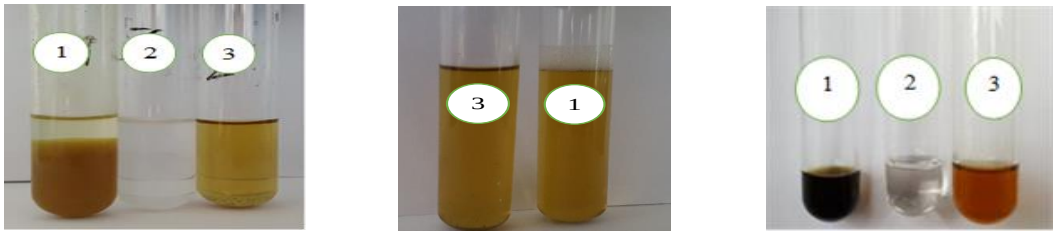
### 3.2. Phytochemical screening of *Bluméa balsamifera* extract

*Bluméa balsamifera* extract contains bioactive compounds including alkaloids, flavonoids, saponins, terpenoids, tannins, and phenol (Table 3, Figure 1). Saponins, flavonoids, and terpenoids have the ability to inhibit the formation and dissolution of calcium oxalate crystals (Saranya & Geetha, 2014).

**Table 3. Bioactive compounds present in the extract**

| Bioactive compound | Results |
|---|---|
| Saponins | + |
| Flavonoids | + |
| Terpenoids | + |
| Alkaloids | + |
| Tannins and phenol | + |
| Steroids | - |

Note: '+': positive and '-': negative.




Flavonoid Saponin Terpenoid

**Figure 1. Bioactive compounds present in the extract**

Note: 1) Sample and reagent; 2) Water and reagent; 3) Extract and water.

### 3.3. Nucleation assay

Citrate is a potential inhibitor for calcium oxalate stones because it forms a complex with calcium, so the concentration of calcium oxalate will be decreased. In the presence of citrate, the aggregation inhibition of urine macromolecules will be increased, and the expression of urinary osteopontin, an important component of the protein matrix of kidney stones, will be reduced (Gupta et al., 2011). Therefore, the inhibition activity of citrate was used as a control to compare with that of extract.

The highest inhibition percentage in the nucleation assay was 88.10 ± 0.14% at 10.00 mg/ml of trisodium citrate, and the lowest inhibition percentage was 0.00% for the distilled water control sample (Table 4). The $IC_{50}$ value of sodium citrate for inhibition in the nucleation assay was 1.16 mg/ml. The highest inhibition percentage in the nucleation assay was 67.25 ± 0.15% at 10.00 mg/ml of extract, and the lowest inhibition percentage was 0.00% for the distilled water control sample (Table 5). The $IC_{50}$ value of *Bluméa balsamifera* extract for inhibition in the nucleation assay was 4.25 mg/ml.

**Table 4. Results of nucleation, growth, and aggregation inhibition of sodium citrate**

| Concentration (mg/ml) | Percent of nucleation (%) | Percent of growth (%) | Percent of aggregation (%) |
|---|---|---|---|
| 0.00 | $0^{l}$ | $0^{l}$ | $0^{l}$ |
| 0.25 | $30.42^{k} \pm 0.39$ | $29.29^{k} \pm 0.56$ | $20.60^{k} \pm 0.65$ |
| 0.50 | $39.48^{h} \pm 0.51$ | $39.84^{h} \pm 0.51$ | $35.13^{h} \pm 0.14$ |
| 0.75 | $45.42^{g} \pm 0.42$ | $44.55^{g} \pm 0.55$ | $40.11^{g} \pm 0.22$ |
| 1.00 | $49.55^{f} \pm 0.57$ | $54.10^{f} \pm 0.43$ | $44.77^{f} \pm 0.71$ |
| 2.00 | $53.47^{e} \pm 0.47$ | $59.70^{e} \pm 0.56$ | $49.99^{e} \pm 0.80$ |
| 4.00 | $65.16^{d} \pm 0.50$ | $65.15^{d} \pm 0.22$ | $53.32^{d} \pm 0.23$ |
| 6.00 | $75.29^{c} \pm 0.46$ | $69.98^{c} \pm 0.12$ | $57.18^{c} \pm 0.47$ |
| 8.00 | $80.62^{b} \pm 0.48$ | $75.29^{b} \pm 0.15$ | $60.47^{b} \pm 0.36$ |
| 10.00 | $88.10^{a} \pm 0.14$ | $79.97^{a} \pm 0.21$ | $63.61^{a} \pm 0.13$ |

Note: In the same column**,** the values followed by the same character do not differ significantly by 5%.

**Table 5. Results of nucleation, growth, and aggregation inhibition of *Bluméa balsamifera* extract**

| Concentration (mg/ml) | Nucleation inhibiton (%) | Growth inhibition (%) | Aggregation inhibition (%) |
|---|---|---|---|
| 0.00 | $0^{l}$ | $0^{l}$ | $0^{l}$ |
| 0.25 | $15.21^{k} \pm 0.15$ | $13.58^{k} \pm 0.05$ | $14.53^{k} \pm 0.11$ |
| 0.50 | $28.24^{h} \pm 0.16$ | $20.33^{h} \pm 0.31$ | $28.42^{h} \pm 0.19$ |
| 0.75 | $34.88^{g} \pm 0.23$ | $21.49^{g} \pm 0.19$ | $34.23^{g} \pm 0.08$ |
| 1.00 | $41.26^{f} \pm 0.07$ | $39.26^{f} \pm 0.08$ | $41.96^{f} \pm 0.21$ |
| 2.00 | $45.16^{e} \pm 0.09$ | $46.32^{e} \pm 0.22$ | $49.65^{d} \pm 0.13$ |
| 4.00 | $49.82^{d} \pm 0.12$ | $56.27^{c} \pm 0.25$ | $57.48^{b} \pm 0.08$ |
| 6.00 | $53.93^{c} \pm 0.30$ | $62.85^{b} \pm 0.05$ | $61.19^{a} \pm 0.06$ |
| 8.00 | $60.41^{b} \pm 0.22$ | $70.82^{a} \pm 0.10$ | $55.44^{c} \pm 0.14$ |
| 10.00 | $67.25^{a} \pm 0.25$ | $51.89^{d} \pm 0.23$ | $47.35^{e} \pm 0.25$ |

Note: In the same column, the values followed by the same character do not differ significantly by 5%.

The inhibitory effects on calcium oxalate crystallization were assessed using $IC_{50}$ values. As a result, the $IC_{50}$ value of 4.25 mg/ml for the extract was higher than the $IC_{50}$ of 1.16 mg/ml for sodium citrate (Table 6) because sodium citrate can directly react with calcium ions and immediately reduce the crystal density (Gupta et al., 2011). Nirmaladevi et al. (2012) found that *Hibscus rosa-sinensis* Linn flower extract inhibited nucleation by approximately 30.00% at a concentration of 1.40 mg/ml. Agarwal and Varma (2015) found that *Achyranthes aspera* L. extract inhibited nucleation by approximately 60.06 ± 0.19% at a concentration of 1.00 mg/ml. Trần (2016) found that *Carmona microphylla* L. extract was an inhibitor in the nucleation assay of calcium oxalate with a value of $IC_{50}$ = 1.76 mg/ml.

**Table 6. Comparison of the $IC_{50}$ value of calcium oxalate crystallization by sodium citrate and *Bluméa balsamifera* extract**

| No. | Stage of inhibition of COM | $IC_{50}$ (mg/ml) | |
|---|---|---|---|
| | | Sodium citrate | *Bluméa balsamifera* extract |
| 1 | Nucleation | 1.16 | 4.25 |
| 2 | Growth | 0.89 | 2.99 |
| 3 | Aggregation | 2.68 | 2.56 |

*Bluméa balsamifera* extract of ethanol has the ability to inhibit the formation of calcium oxalate stones. COD crystals have a lower affinity for cell membranes than COM crystals, so it is difficult for them to attach to cell membranes, causing kidney stones that are easily eliminated through the urinary tract and do not cause damage to the urinary system. COM crystals can be converted to COD crystals or reduced in density due to the presence of natural compounds of the flavonoid group (Agarwal & Varma, 2015).

Therefore, low density COM crystals appear in the sample due to the flavonoid, saponin, and terpenoid compounds contained in the extract.

### 3.4. Growth assay

The highest inhibition percentage in the growth assay was 79.97 ± 0.21% at 10.00 mg/ml of sodium citrate, and the lowest inhibition percentage was 0.00% for the distilled water control (Table 4). The $IC_{50}$ value of sodium citrate for inhibition in the growth assay was 0.89 mg/ml. The highest inhibition percentage in the growth assay was 71.62 ± 0.44% at 8.00 mg/ml of extract, and the lowest inhibition percentage was 0.00% for the distilled water control (Table 5). The $IC_{50}$ value of *Bluméa balsamifera* extract for inhibition in the growth assay was 2.99 mg/ml.

The inhibitory effects of calcium oxalate crystallization were assessed by $IC_{50}$ values. As a result, the $IC_{50}$ value of 2.99 mg/ml for the extract was higher than the $IC_{50}$ of 0.89 mg/ml for sodium citrate (Table 6) because sodium citrate can directly react with calcium ions and immediately reduce the crystal density (Gupta et al., 2011). Trần (2016) found that *Carmona microphylla* L. extract inhibited calcium oxalate in a growth assay with a value of $IC_{50}$ = 1.50 mg/ml.

*Bluméa balsamifera* extract can inhibit the growth of calcium crystals by coating the surface of the crystals. Moreover, the bioactive compounds can interact and form a complex with calcium and oxalate ions to inhibit the growth of the crystals (de Cógáin et al., 2015). When the concentration of the extract reaches 10.00 mg/ml, the inhibition percentage decreases because saponins and flavonoids can both transfer COM to COD, increasing the formation of COD so the extract can easily pass through the renal tube (Atmani & Khan, 2000).

### 3.5. Aggregation assay

The highest inhibition percentage in the aggregation assay was 63.61 ± 0.13% at 10.00 mg/ml of sodium citrate, and the lowest inhibition percentage was 0.00% for the distilled water control sample (Table 4). The $IC_{50}$ value of sodium citrate for inhibition in the aggregation assay was 2.68 mg/ml. The highest inhibition percentage in the aggregation assay was 72.19 ± 0.09% at 6 mg/ml of extract, and the lowest inhibition percentage was 0.00% for the distilled water control sample (Table 5). The $IC_{50}$ value of *Bluméa balsamifera* extract for inhibition in the aggregation assay was 2.56 mg/ml.

The inhibitory effects on calcium oxalate crystallization were assessed by $IC_{50}$ values. The $IC_{50}$ value of 2.68 mg/ml for sodium citrate was higher than that of the extract $IC_{50}$, which was 2.56 mg/ml (Table 6). The reason is that sodium citrate has the ability to strongly inhibit the development of calcium oxalate stones by creating salts dissolved with oxalate ions, while in the calcium oxalate stone condensation stage, the condensation inhibiting effect is lower (Gupta et al., 2011). This result is higher than that of Vyawahare et al. (2014), who found that an extract of *Momordica charantia* Linn. inhibited 30.00% of crystal aggregation at 0.50 mg/ml, and the results of Agarwal and Varma (2015), who

found that a 100.00% *Ocimum gratissimum* L. extract inhibited 62.07% of crystal aggregation. Trần (2016) found that *Carmona microphylla* L. extract was an inhibitor of calcium oxalate in an aggregation assay with $IC_{50}$ = 0.80 mg/ml.

Aggregation is a critical step in the stone formation process in which small crystals form a cluster by strong chemical or electrical forces and increase in size (Nirmaladevi et al., 2012). The extract *Bluméa balsamifera* had the highest inhibition percentage because the bioactive compound coated the surface of the crystals to prevent aggregation (Pareta et al., 2011). Moreover, saponins and terpenoids can inhibit crystal aggregation by interacting with mucoprotein, the primary factor causing the supersaturation of calcium oxalate crystals, which initiates the stone formation process (Saha & Verma, 2013). However, at 8.00 mg/ml and 10.00 mg/ml, the inhibitory percentage was lower than 6.00 mg/ml, because the extract promotes the formation of COD. COD can easily be extracted through the renal tubes without any risk from the kidney stone (Atmani & Khan, 2000). The $IC_{50}$ index of the extract (2.56 mg/ml) was lower than that of sodium oxalate (2.68 mg/ml) because the main inhibitory effect of sodium citrate is focused on the growth phase (Gupta et al., 2011).

The results of investigating the ability to inhibit the formation of crystals of calcium oxalate in the three main phases, nucleation, growth, and aggregation, were $IC_{50}$ values of 4.25, 2.99, and 2.56 mg/ml, respectively. The research results show that the extract of the compassion plant has the ability to inhibit the formation of crystals of calcium oxalate, which is also consistent with the study of Charlimagne and Rizalinda (2017) that the extract of the compassion plant is capable of reducing the size of calcium oxalate crystals with a dosage of extract of 0.50 mg/mL and 1.00 mg/ml. Research results show that compassion plant extract can be a raw material source for products to treat diseases, especially diseases related to kidney stones.

## 4. CONCLUSIONS

*Bluméa balsamifera* extract has a significant inhibitory effect on the nucleation, growth, and aggregation of calcium oxalate crystallization, which causes kidney stones. Further study on *Bluméa balsamifera* extract on the in vivo model of calcium oxalate inhibition. Separation and purification of bioactive compounds from *Bluméa balsamifera* have the potential to treat kidney stones.

## ACKNOWLEDGMENTS

Thanks to An Giang Biotechnology Center and An Giang Department of Science and Technology for facilitating and supporting this research.

## REFERENCES

Agarwal, K., & Varma, R. (2015). In-vitro calcium oxalate crystallization inhibition by *Achyranthes aspera* L. and *Bryophyllum pinnatum* Lam. *British Journal of Pharmaceutical Research, 5*(2), 146-152.

Atmani, F., & Khan, S. R. (2000). Effects of an extract from Herniaria *hirsuta* on calcium oxalate crystallization in vitro. *BJU International*, *85*(6), 621-625.

Atodariya, U., Barad, R., Upadhyay, S., & Upadhyay, U. (2013). Anti-Urolithiatic activity of *Dolichos biflorus* seeds. *Journal of Pharmacognosy and Phytochemistry*, *2*(2), 209-213.

Bandar, H., Hijazi, A., Rammal, H., Hachem, A., Saad, Z., & Badran, B. (2013). Techniques for the extraction of bioactive compounds from Lebanese *Urtica dioica*. *American Journal of Phytomedicine and Clinical Therapeutics*, *6*, 507-513.

Charlimagne, M. M., & Rizalinda, L. D. L. (2017). Effect of Blumea *balsamifera* extract on the phase and morphology of calcium oxalate crystals. *Asian Journal of Urol*ogy, *4*(4), 201-207.

Chaudhary, A., Singla, S. K., & Tandon, C. (2010). In vitro evaluation of *Terminalia arjuna* on calcium phosphate and calcium oxalate crystallization. *Indian Journal of Pharmaceutical Sciences*, *72*(3), 340-345.

de Cógáin, M. R., Linnes, M. P., Lee, H. J., Krambeck, A. E., de Mendonça Uchôa, J. C., Kim, S. H., & Lieske, J. C. (2015). Aqueous extract of *Costus arabicus* inhibits calcium oxalate crystal growth and adhesion to renal epithelial cells. *Urolithiasis*, *43*(2), 119-124.

Gupta, M., Bhayana, S., & Sikka, S. K. (2011). Role of urinary inhibitors and promoters in calcium oxalate crystallisation. *International Journal of Research in Pharmacy and Chemistry*, *1*(4), 793-798.

Nirmaladevi, R., Kavitha, D., & Padma, P. R. (2012). Evaluation of antilithiatic potential of *Hibiscus rosa-sinensis* Linn, in vitro. *Journal of Pharmacy Research*, *5*(8), 4353-4356.

Pareta, S. K., Prata, K. C., & Ranjit, K. H. (2011). In-vitro calcium oxalate crystallization inhibition by *Achyranthes indica Linn*. Hydroalcoholic extract: An approach to antilithiasis. *International Journal of Pharma and Bio Sciences*, *2*(1), 432-437.

Phatak, R. S., & Hendre, A. S. (2015). In-vitro antiurolithiatic activity of *Kalanchoe pinnata* extract. *International Journal of Pharmacognosy and Phytochemical Research*, *7*(2), 275-279.

Saha, S., & Verma, R. J. (2013). Inhibition of calcium oxalate crystallisation in vitro by an extract of *Bergenia ciliata*. *Arab Journal of Urology*, *11*(2), 187-192.

Saranya, R., & Geetha, N. (2014). Inhibition of calcium oxalate (CaOx) crystallization in vitro by the extract of beet root (*Beta vulgaris* L.). *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(2), 361-365.

Trần, Đ. T. (2016). *Ảnh hưởng của dịch trích lá và thân cây Bùm sụm (Carmona microphylla L.) lên sự ức chế hình thành tinh thể Calcium oxalate gây bệnh sỏi thận trong điều kiện in vitro* (Bachelor's thesis, Can Tho University, Vietnam).

Vyawahare, J. N., Shelke, P. A., Aragade, P. D., & Baheti, D. G. (2014). Inhibition of calcium oxalate crystallization in vitro by extract of *Momordica charantia* Linn. *International Journal of Pharmaceutical and Chemical Sciences, 3*(2), 448-452.

Yadav, M., Chatterji, S., Gupta, S. K., & Watal, G. (2014). Preliminary phytochemical screening of six medicinal plants used in traditional medicine. *International Journal of Pharmacy and Pharmaceutical Sciences, 6*(5), 539-542.