Effect of *Blumea Balsamifera* Extract on the Kinetics of Calcium Oxalate Monohydrate (COM) Dissolution

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***Abstract*—*Blumea balsamifera* is a commercially available herbal drug that has anti-urolithic and diuretic properties. This study quantifies the effect of *B. balsamifera* extract on the dissolution kinetics of Calcium Oxalate Monohydrate (COM) crystals by modeling the dissolution of Ca^{2+}. COM dissolution follows a first-order diffusion-controlled process. The extract did not change the dissolution phenomena. At 10 ppm, the extract had no significant effect on the rate constant ($P = 0.166$) and surface concentration ($P = 0.372$). Increasing the extract to 20 ppm did not change the dissolution phenomena and model parameters. The extract significantly decreases the equilibrium Ca^{2+} concentration with $P = 0.0010$ and $P = 0.0179$ at 10 and 20 ppm of extract, respectively. *B. balsamifera* extract binds free Ca^{2+} in the synthetic urine. This increases the amount of Ca^{2+} that dissolves but does not significantly increase the rate of dissolution suggesting that urine volume is more important for COM stone dissolution.**

***Index Terms*—*Blumea balsamifera* extract, dissolution kinetics, ethanol extract, kidney stones**

I. Introduction

Calcium stones are the most common type of kidney stones, containing up to 75% Calcium Oxalate Monohydrate (COM) crystals in human kidney stones [1]. *Blumea balsamifera*, or Sambong is an anti-urolithic and diuretic drug, currently being sold in the market. It prevents the formation of urinary stones and increases the rate of urine flow. Various phytochemicals were isolated from the extracts of *B. balsamifera* leaves. These are generally classified as terpenes, terpenoids, sesquiterpenoids and flavonoids [2, 3]. Pharmacokinetic studies established that flavonoids are eliminated from the body through urinary excretion [4] and this may explain the therapeutic effect of *B. balsamifera* on kidney stones. Studies on *B. balsamifera* showed that the extract favours formation of the less pathogenic calcium Oxalate Dihydrate (COD) over COM [5] while an Overall decrease in number of stones was reported in Wistar rats [6]. Rico studied the dissolution of calcium stones in the presence of urine with *B. balsamifera*. Fresh urine samples from individuals who consumed 0, 40, and 60 mg/kg/day of *B. balsamifera* tablets for ten days were collected and were used to dissolve the calcium stones. The study found that all three levels of dosage had a statistically significant effect on kidney stones dissolution with 40 mg/kg/day as the best level of dosage [7]. Vinco and Sunga also studied the effect of *B. balsamifera* extract on the dissolution of kidney stones. They found that, after 72 h, calcium oxalate stones had a weight loss of 13% at an average rate of 0.001 g/h [8]. The studies by Rico and by Vinco and Sunga showed that *B. balsamifera* is beneficial for dissolving kidney stones but does not explain the mechanism.

Though it had been shown that *B. balsamifera* decreases kidney stone formation, its effect on the kinetics of kidney stones dissolution has not been studied. In this study, the effect of *B. balsamifera* extract on the dissolution kinetics of COM crystals will be determined using Ca^{2+}-specific electrode. By establishing the role of *Blumea balsamifera* in COM dissolution, better formulation of *B. balsamifera*-based drugs for kidney stone treatment can be created.

Manuscript received May 30, 2023; revised June 25, 2023, accepted August 3, 2023.

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II. Materials and Methods

A. Preparation of COM Crystals

The crystallization of COM was induced at room temperature according to literature [9]. Stock solutions of calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$, AJAX Finechem, Australia) and sodium oxalate ($Na_2C_2O_4$, AJAX Finechem, Australia) were prepared to crystallise artificial calcium oxalate kidney stones. Stock solutions of 0.03 M $CaCl_2 \cdot 2H_2O$ and 0.1 M $Na_2C_2O_4$ were prepared using deionised water as solvent. After 7 days of crystallization, the COM kidney stones were filtered out using a Whatman filter paper and then dried in a desiccator for 1 day at room temperature.

B. Extract Preparation

An ethanolic extract was prepared by Soxhlet extraction according to literature [10]. *B. balsamifera* leaves were dried at room temperature for at least 4 days, crushed using mortar and pestle, and was extracted using Soxhlet extraction with 95% ethanol (AJAX Finechem, Australia). The extraction process lasted until at least 4 siphons were reached. The ethanol was boiled off, and then the extract was filtered using Whatman filter paper.

C. Dissolution of Stones

Modified synthetic urine was prepared based on literature [11]. Interfering components such as calcium, oxalate, and magnesium were removed from the formulation, so that only the dissolution of COM kidney stones will be taken into consideration. Synthetic urine was prepared immediately before use by mixing equal volumes of solutions A and B, each at 250 mL in a 1.0 L beaker. One mL of 30% H_2O_2 was added to both solutions and were filtered using a Whatman filter paper. The solutions were stored for a maximum of one week at 4 °C. The pH was adjusted to 6.9 at 37 °C and the composition are provided in Table I.

doi: 10.18178/ijcea.2023.14.4.805

TABLE I: COMPOSITION OF SYNTHETIC URINE

Solution A (mol/L)		Solution B (mol/L)	
$Na_2SO_4 \cdot 10H_2O$	0.0342	$NaH_2PO_4 \cdot 2H_2O$	3.4×10^{-5}
NH_4Cl	0.0869	$Na_2HPO_4 \cdot 12H_2O$	0.00011
KCl	0.1625	NaCl	0.2245

Synthetic urine was incubated and maintained at 37 ºC prior to the addition of *B. balsamifera* extract and 0.050 g of COM crystals. The extract concentration was varied from 0 for the control, to 10 and 20 mg dried leaves per liter of the final solution. Eutech® 700 pH/mV/ ºC /ºF Bench Meter with a Ca^{2+} specific electrode (Eutech Instruments Pte Ltd., Singapore) was calibrated and submerged in each constantly stirred solution sample until the measurement remains constant. Data was collected and recorded every 1 min for the first 30 min, every 5 min for the latter half of the first hour, and every 15 min onwards. Least-squares regression was performed to investigate the mechanism of dissolution. The sum of squares of errors was minimised by changing the model parameters, C_s and k for the 1st and pseudo-2nd order models.

III. RESULTS AND DISCUSSION

COM crystals were soaked in synthetic urine and calcium ion concentration profiles were obtained. Three replicates were performed for the extract-free control in synthetic urine, and synthetic urine with 10 and 20 ppm of extract as mg dry leaves per Liter (ppm). Calcium ion concentration reached a constant value after 120 minutes and data collection was stopped. Previous studies modelled the dissolution of COM by 1st and pseudo-2nd order equations. Gardner and Nancollas modelled the dissolution kinetics of COM crystals. They concluded that the dissolution was diffusion controlled as the best fit was obtained using a 1st order model represented by Eq. (1) [12].

$$\ln\left(\frac{c_s - c_o}{c_s - c}\right) = kt \quad (1)$$

where c is the concentration of free calcium ions in the liquid at time t, c_0 at time 0, and c_s is the concentration of the free calcium ions at the crystal surface. The pseudo-2nd order model represents surface-controlled dissolution and is represented by equation 2 where c is the concentration at time t, c_o is the initial concentration, c_s is the surface concentration, and k' is the pseudo-second order dissolution constant [13].

$$\frac{t}{c - c_o} = \frac{1}{k'(c_s - c_o)^2} + \frac{t}{(c_s - c_o)} \quad (2)$$

Least squares regression was performed to assess the fit of the dissolution models. In synthetic urine without the extract, the 1st order model resulted to an acceptable fit with a coefficient of determination (r^2) of 0.894 ± 0.086. However, the pseudo-2nd order model r^2 value is 0.798 ± 0.134, suggesting that this model does not accurately describe the dissolution process. Relative to the extract-free control, the fit of the 1st order model improved at 10 and 20 ppm extract with r^2-values of 0.960 ± 0.017 and 0.976 ± 0.013, respectively. The pseudo-2nd order model had better r^2 values with the addition of the extract but are still far from 1. A summary of the r^2 values for the 1st and pseudo-2nd order models and concentration profiles at different extract concentrations are shown in Figs. 1 and 2.

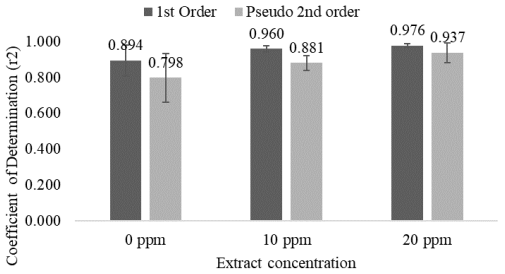

Fig. 1. Summary of r^2 values shows that the first order model consistently gave the best fit of the dissolution data across all levels of extract concentration. Data are mean ± SD, n = 3.

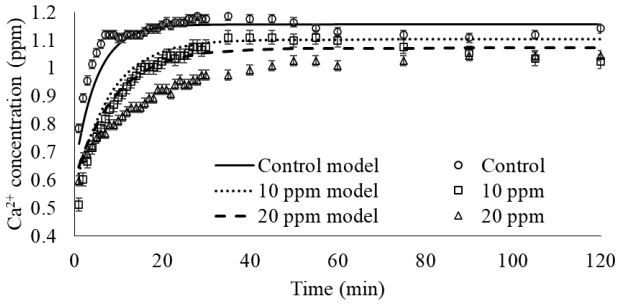

Fig. 2. The first order model predicted and experimental Ca^{2+} concentration profile at different extract concentrations shows an increasing concentration until a constant concentration is achieved. Data points are mean ± SD, n = 3.

White and Nancollas reported that COM dissolution is a first order process at high undersaturation and shifts to pseudo-2nd order when the undersaturation in low. This shift happens at an undersaturation of 0.2 [14]. In this study, the undersaturation ranges from 0.697 to 0.885 which are obtained over the range of observed Ca^{2+} measurements. The conditions used in this study is within the undersaturation range of the first order model, resulting into an acceptable fit that is consistent with other studies of COM dissolution [12, 15].

The first order model was used to further assess the effect of the extract on the dissolution of COM crystals. The model parameters were averaged across the replicate runs. A summary of the parameters is presented in Fig. 3. Student's T-test on the mean surface concentrations (Cs) shows no significant effect of the extract with *P*-values of 0.372 and 0.278 at 10 and 20 ppm extract, respectively. The extract had no significant effect on the dissolution rate constants (k) as Student's T-test resulted to *P*-values of 0.166 and 0.210 at 10 and 20 ppm extract, respectively. B. balsamifera extract does not have a significant effect on the kinetics of COM stone dissolution.

The first order model shows that the dissolution process is diffusion controlled. When diffusion predominates, compounds that inhibit crystallization by surface adsorption will not affect the dissolution [12]. Though components of *B. balsamifera* extract were not directly shown to bind the surface of COM crystals, studies showed that the extract influence crystallization by shifting crystal morphology from COM to COD which suggests surface interactions [5]. As

predicted from the study of Garder and Nancollas, the extract components had no effect on the dissolution process.

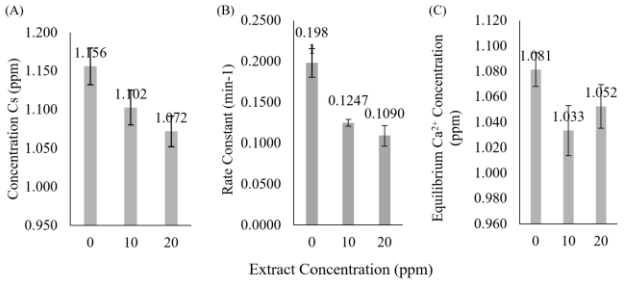

Fig. 3. (a) Increasing the extract concentration decreases the model parameters surface concentration (c_s) and (b) the dissolution rate constant. (c) Student's T-test showed that the equilibrium calcium concentration was significantly lower at 10 and 20 ppm, with P = 0.0010 and P = 0.0179, respectively. Data points are mean ± SD, n = 3.

Concentration data beyond 90 minutes were averaged to obtain equilibrium Ca^{2+} concentration as shown in Fig. 3(c). Statistical analysis showed a significant difference in equilibrium Ca^{2+} concentration at 10 and 20 ppm extract with P = 0.0010 and P = 0.0179, respectively. This suggests that the extract influences the equilibrium Ca^{2+} concentration.

The device used to measure Ca^{2+} concentration is an Ion-Specific Electrode (ISE). The electrode contains a membrane that develops a potential difference in response to Ca^{2+} that bind to the surface. Plant extracts were shown to influence dissolution by binding with calcium ions. Das et al. attributed the effect of *Trianthema monogyna* extract to its interaction with Ca^{2+}. In their study, UV-vis and IR spectroscopy showed changes in the spectra suggesting the chelation of calcium ions by the plant extracts [16]. The abundance of hydroxyl groups in a compound were shown to increase the interaction of organic compounds with calcium ions [17]. Bound calcium ions are unable to interact with the Ca-ISE, effectively reducing the detected ion concentration. Thus, the amount of dissolved COM crystals is higher than what is being reflected on the measurements. To test for Ca^{2+}-extract binding, the concentration of standard Ca^{2+} solution was measured before and after the addition of the extract to quantify the effect of extract addition. Adding the extract to a concentration of 10 ppm decreased Ca^{2+} concentration by 5.76%. At 20 ppm, the decrease is 2.94%. The difference represents the extract bound Ca^{2+}. When the sum of the bound Ca^{2+} and equilibrium Ca^{2+} concentration is taken, the extract no longer has an effect with P-values of 0.8175 and 0.9680, at 10 and 20 ppm extract respectively. Calcium binding test is summarized in Table II and Fig. 4.

Thermodynamics predict that further dissolution should occur in the undersaturated synthetic urine environment and temperature used. However, the kinetics of the process is at a pseudo equilibrium state. Dissolution processes reach a pseudo equilibrium state when surface rearrangement occurs on the crystals [18]. The dissolution kinetics considered in this study was limited by the pseudo equilibrium state encountered. Other equilibrium studies considered dissolution over a period of 5 [17], 10 [7], and 20 days [16]. Despite the relatively shorter observation period, the initial dissolution rate obtained in this study is 1.103 ± 0.229 mg/hr and is comparable with the average dissolution rate reported in literature at 1 mg/hr [8]. Addition of the extract changed the initial dissolution rate to 1.078 ± 0.095 and 0.698 ± 0.140 at 10 and 20 ppm of extract, respectively. Student's T-test shows that the differences are not statistically significant (P > 0.05).

TABLE II: CALCIUM BINDING TEST SHOWS A DECREASE IN Ca^{2+} CONCENTRATION AFTER THE ADDITION OF THE EXTRACT

Extract	[Ca^{2+}] Before	[Ca^{2+}] After	%Difference
10 ppm	1.872	1.764	−5.762
20 ppm	2.006	1.948	−2.924

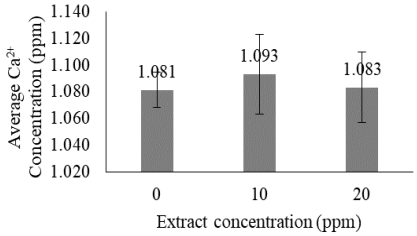

Fig. 4. When the sum of the equilibrium Ca^{2+} concentration and the %difference from bound Ca^{2+} was added, there is no significant difference on the Ca^{2+} concentration at 10 and 20 ppm with P-value of 0.8175 and 0.9680, respectively. Data are mean ± SD, n = 3.

This study showed that *B. balsamifera* extract does not significantly influence the kinetics of COM dissolution, but the extract components interact with Ca^{2+} which increase the total amount of dissolved Ca^{2+} in synthetic urine. This was consistent with a previous study involving *B. balsamifera* leaves [7] and for other plant extracts [16]. As Ca^{2+} are bound in the extract, the driving force for the first order dissolution increase. However, this increase is only up to 5% and may even be negligible depending on the relative amount of the surface and equilibrium concentrations. COM stone dissolution rate may however be increased by combining the presence of the extracts with a high urine volume. As the extract increases the amount of dissolved Ca^{2+} in the urine and more urine is expelled, more Ca^{2+} are removed from solid COM.

IV. CONCLUSION

The dissolution of COM crystals in synthetic urine is a diffusion controlled first order process. *B. balsamifera* extract had no significant effect on the dissolution rate constant and it does not increase the overall rate of dissolution. However, the extract significantly decreases the equilibrium concentration. Further analysis showed that the extract components interact with calcium ions which explains previous reports of increased overall calcium concentration. However, this increase in calcium is not big enough to significantly change the dissolution rate.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed equally in this study. CMontealegre conceptualized the topic, analyzed data and wrote the final paper. LNChan, SVPeralta and SESo conducted the experiment analyzed the data, and wrote parts

of the paper. All authors approved the final version.

FUNDING

This study was supported by UP Engineering Research & Development Foundation Inc. (Philippines).

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