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In vitro mass reduction of calcium oxalate urinary calculi by some medicinal plants

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Abstract

Background: Calcium oxalate is the most frequent urinary stone component; it exists in three different crystalline forms. In this case, the most common is the calcium oxalate monohydrate (whewellite). However, Morocco is one of the countries that has used the traditional medicine based on natural plants to treat many diseases including urolithiasis. In this respect, the most medicinal plants used for this purpose are *Herniaria hirsuta* L., *Opuntia ficus-indica* flowers, *Zea mays* styles and *Ammi visnaga* L. seeds. The purpose of this work is to study experimentally the effectiveness of each plant on the dissolution of whewellite stones.

Methods: In 1 L boiled saline solution (9 g/L of NaCl), 5 g of plant extract powder has been introduced. Thereafter, the mixture was left soaked for 15 min and then filtered. Further, a specific installation that resembles the urinary circuit has been conducted in the laboratory. As a starting step, three whewellite stones are placed in contact with extract solutions for 8 weeks. Two other solutions have been used to correct the loss mass: the first one is a solution of potassium citrate of 3 mmol/L served as a positive control, and the second one is a solution of NaCl of 9 g/L without extract used as a reference point.

Results: After 8 weeks, the loss mass is about $54.88 \pm 1.32\%$ with *Z. mays* styles, $49.86 \pm 1.69\%$ with *H. hirsuta* L., $47.10 \pm 10.19\%$ with *A. visnaga* L. and $44.97 \pm 1.76\%$ with flowers *O. ficus-indica*, while the loss of mass in the presence of witnesses solutions is $21.95 \pm 0.76\%$ for potassium citrate (C Pot) and $21.05 \pm 1.07\%$ in the case of saline solutions.

Conclusion: Our experiments show the effectiveness of the extracts of four plants specially for *Z. mays* styles. These extracts can be effective remedies in the oxalocalcic stones' dissolution.

Keywords: Nephrolithiasis, Medicinal plants, Urinary stones, Potassium citrate, Dissolution rate

1 Background

Urolithiasis is a common disease that evolves with the socio-economic and health status of populations. In this regard, it is characterized by crystalline concretion formation in the urinary tract, and it is a widespread illness in the active population. Therefore, it affects nearby 4–20% of the general population of different countries. However, it is readily recurrent and its etiology is often unknown [1]. On the other hand, calcium oxalate is the most abundant element, its prevalence exceeds 70% in

most of world's countries [2, 3], and the monohydrate form is oxalo-dependent crystallizing in environments of hyperoxaluria with low or normal calcium. However, to our knowledge there are no effective drugs for the dissolution of calcium oxalate monohydrate stones; in addition, surgical methods are usually invasive with side effects. In recent years, the introduction of expulsive therapy based on drugs able to facilitate the passage of distal ureteral calculi such as nifedipine and tamsulosin, but despite their widespread use, the evidence for the benefit of these agents in the treatment of ureteral calculi remains weak [4–6]. In this respect, several medicinal plants have been used traditionally for the treatment of urinary stones such as *Herniaria hirsuta* L., *Opuntia ficus-indica*, *Zea mays* and *Ammi visnaga* L. These plants

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have often shown the spectacular therapeutic results [7–9]. The aim of this study is to evaluate experimentally the efficiency of some Moroccan medicinal plants for dissolution urinary stones. For this reason, the choice has been focused on calcium oxalate because of both its high prevalence and its extracorporeal lithotripsy resistance.

2 Methods

2.1 The urinary calculi

Calcium oxalate is a calcium salt of oxalic acid with the chemical formula CaC_2O_4 . A major constituent of human kidney stones are formed by calcium oxalate. Various kidney stones of calcium oxalate (Fig. 1) were removed by surgery in an elderly patient of 55 years old suffering from oxalo-dependent lithiasis. These stones whose mass was initially between 125 and 260 mg (Table 1) have a morphology type “Id”, which is characterized by a smooth surface of brown color, and the cross section is compact, with a microcrystalline concentric structure in very thin layers without radial crystallization [10]. Their chemical composition has been analyzed by infrared spectroscopy (Fig. 2).

2.2 Medicinal plants

In this work four medicinal plants are used, the first one is *H. hirsuta* L., fully used (leaves and stems), it is an annual herb with stems up to 20 inches long and collected from the Taza region of North-Eastern Morocco. The second and third ones are *O. ficus-indica* flowers and *A. visnaga* L. seeds. They are collected from the Taounate region of northern Morocco. The fourth one is the very fine filaments that come from the outer shell corn cobs *Z. mays* styles. It is from the region of Fez in northern Morocco. The taxonomic identification was performed by Prof. A. Bari, Department of Biology, Faculty of Sciences Dhar El-Mahraz, Sidi Mohammed Ben Abdellah University,

Table 1 The initial average stones weight

Solution type	W_i
OFl	190 ± 30 mg
HHL	165 ± 28 mg
AVL	160 ± 20 mg
ZMS	125 ± 18 mg
S phy	260 ± 15 mg
C pot	245 ± 21 mg

Fez, Morocco. Voucher specimens were deposited in the herbarium under the following references: RAB 090814; RAB 1264407; RAB 76986 and RAB 1108434, respectively. Then, the four plants were dried at room temperature, ground and stored until the extraction.

2.3 Extraction process

In 1L of physiological saline solution (9 g/L NaCl) boiled, 5 g of the plant powder has been introduced, and the mixture was left soaked for 15 min and then filtered in three steps (Fig. 3). The first via a sieve with port diameter of 125 μm, followed by filtration through filter paper with port diameter of 20 to 25 μm and then through filter paper with port diameter from 7 to 10 μm. The outcome of the plant extracts was compared to an aqueous solution of potassium citrate to 3 mmol/L [8], which corresponds to the average concentration of urinary citrate obtained during the treatment of whewellite, and an aqueous NaCl solution at 9 g/L was used as a witnesses solution.

2.4 Experimental device

A specific installation that resembles the urinary circuit has been done in the laboratory (Fig. 4). Indeed, the solution of the plant extract is placed in a large tank and

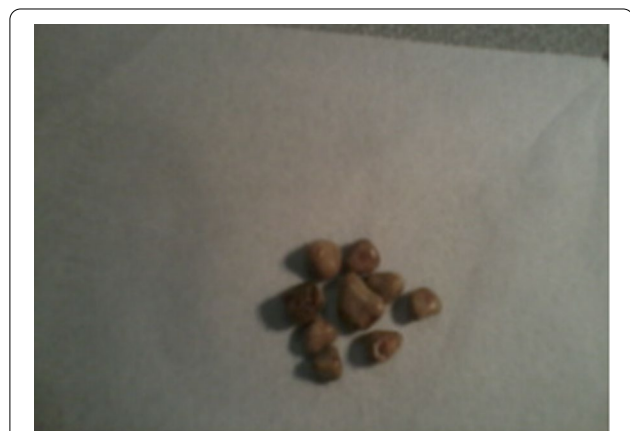


Fig. 1 Kidney stones of calcium oxalate

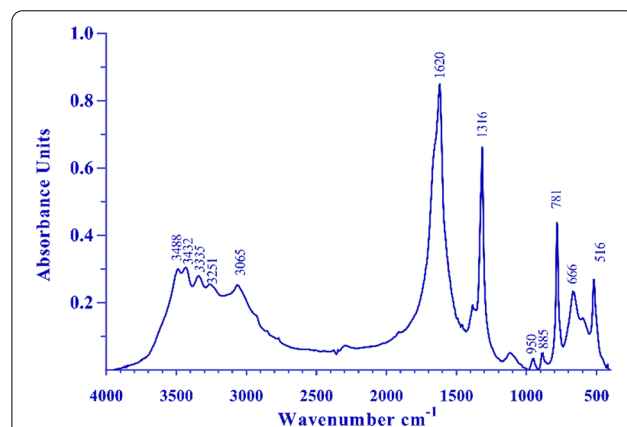


Fig. 2 Infrared spectrum of calcium oxalate

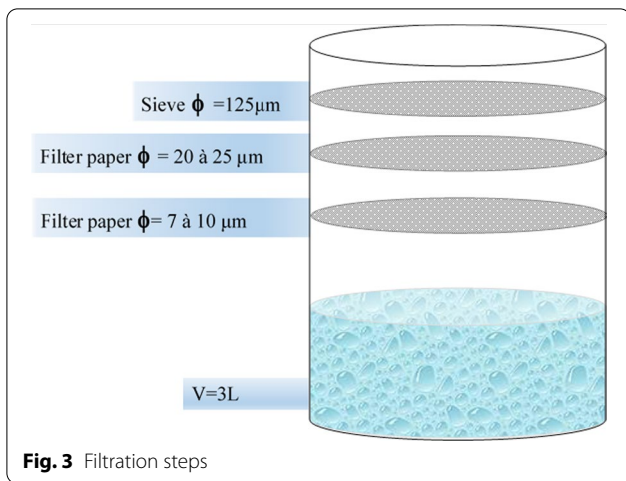


Fig. 3 Filtration steps

whewellite stones are placed in an enclosure using the sample holder, attached at both ends by two tubulars. The first one is to pump the solution from the large tank, and the second one is attached to a second collecting vessel. In this respect, the stones undergo regular flow from the tank through two valves placed upstream and downstream of the chamber for controlling the flow rate of the solution at 1.5 mL/min (2L/day): average urine flow is recommended for the lithiasis patients. Meanwhile, the pH value of the recovered solution is monitored daily using a pH meter. Thereafter, the solution is filtered using a sieve with port diameter of 125 μm and given to the initial tank. Further, the operation lasted for 8 weeks, period recommended in traditional medicine. Hence, every 2 weeks, the stones are removed, dried for 16 h at a temperature of 40 °C and weighed with a precision balance to

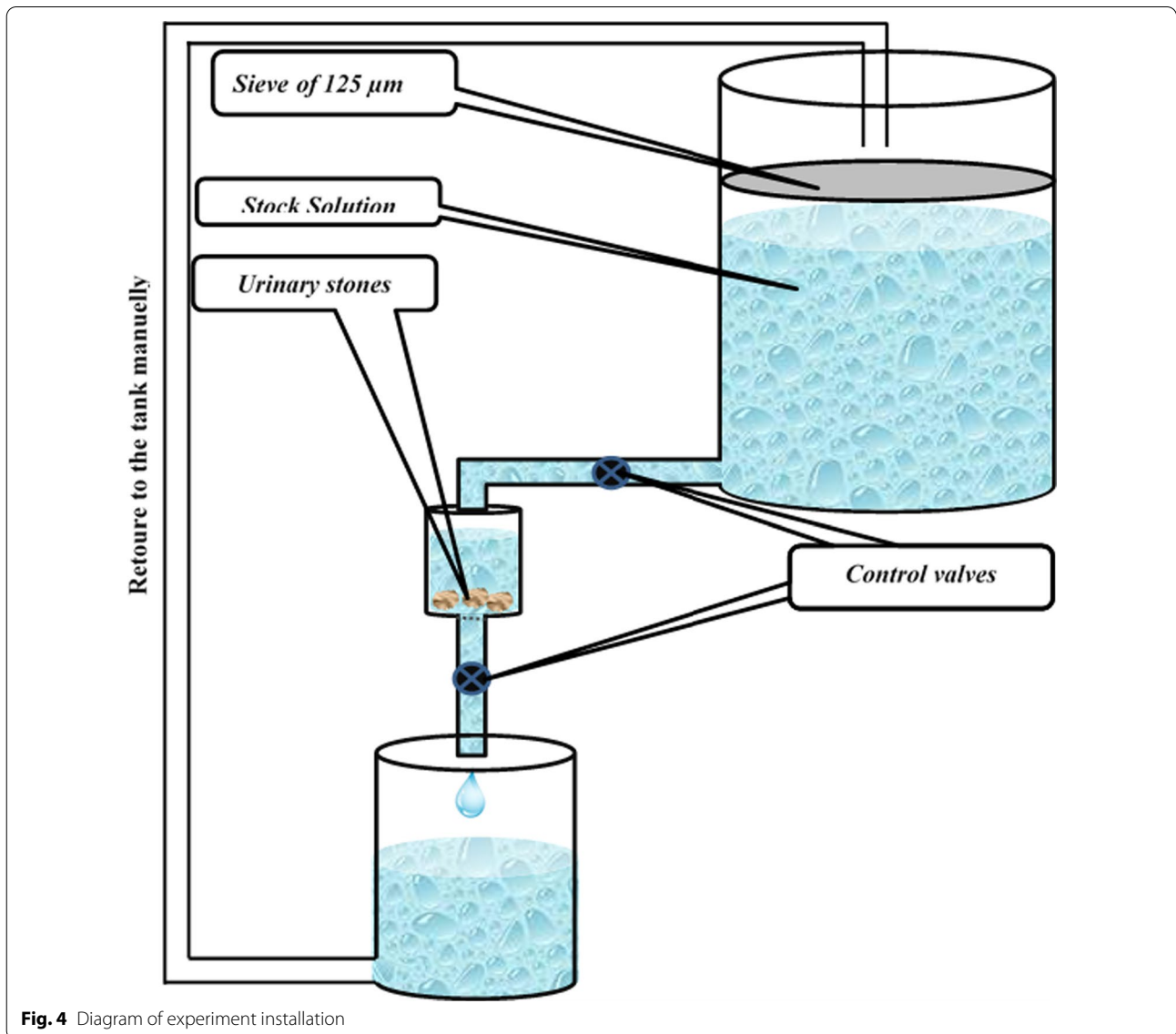


Fig. 4 Diagram of experiment installation

measure the mass loss and then delivered to the infrared spectroscopy to determine their composition. The same procedure was carried out for two other witnesses' solutions to correct the mass loss (Dissolution Rate): The first is a solution of potassium citrate 3 mmol/L, and the second is a saline solution of NaCl 9 g/L [8, 11].

3 Results

3.1 The effect of plant extracts on stones

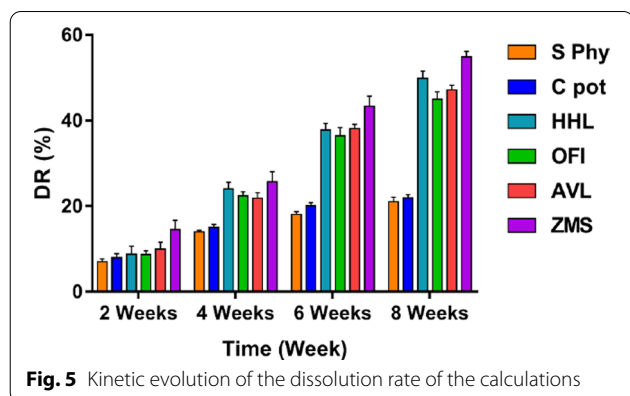
The results provided, expressed as the dissolution rate (DR), were calculated by the following formula and expressed as a mean ± standard deviation

$$(DR\%) = \frac{W_i - W_f}{W_i} * 100$$

with W_i : initial stones weight, W_f : final stones weight.

The initial stones weights " W_i " are given in Table 1.

The analysis of the histogram (Fig. 5) shows a continuous increasing kinetic evolution of DR for the different solutions over the 8 weeks. For this reason, this trend is more important for the all extract solutions than the control solutions. The comparison of DR for the four plants displays that *Zea mays* styles have a slightly greater impact than the others. Indeed, after 2 weeks, this plant has given a DR of about $14.43 \pm 2.22\%$, compared to $9.94 \pm 1.63\%$ for seeds of *Ammi visnaga* L., $8.76 \pm 1.23\%$ for the *H. hirsuta* L. plant, and $8.69 \pm 0.87\%$ for flower extracts of *O. ficus-indica*. Similarly citrate and physiological saline solutions have provided a DR of 7.02 ± 0.65 and $7.93 \pm 1.01\%$, respectively. The same result has been observed after contact of 8 weeks with the plant extracts. Thereby, the DR of $54.88 \pm 1.32\%$ was obtained with *Z. mays* styles extracts and the DR values of 49.86 ± 1.69 , 47.10 ± 1019 and $44.97 \pm 1.76\%$ were found with plant *Herniaria hirsute* L, seeds of *Ammi visnaga* L. and flowers of *O. ficus-indica*, respectively, while with citrate and physiological solutions, the dissolution rates after 8 weeks were 21.95 ± 0.76 and $21.05 \pm 1.07\%$, respectively.



3.2 Kinetic evolution of pH for the four plants

Figure 6 exhibits that the initial pH of the solution is slightly acidic or basic. Thus, it ranges from 6 for the extract of *ficus-indica* flowers to 7.8 for the citrate solution. However, the pH values have slightly increased linearly during treatment time to the values of 7.5 and 9 for *Hirnia Hirustat*, potassium citrate and physiological solutions.

3.3 Proposed mechanisms of action.

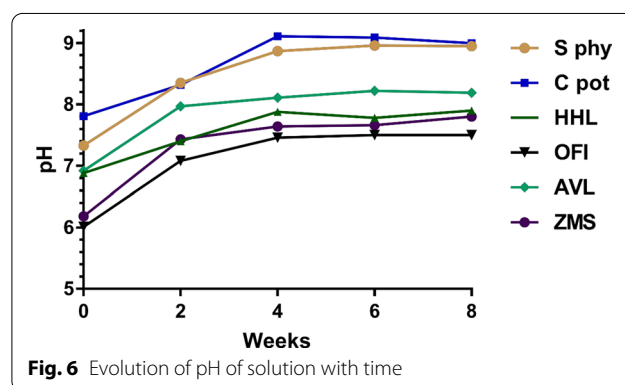
Figure 7 shows the mechanisms of action proposed to explain the interactions between the calcium oxalate molecule and the active ingredient of medicinal plants.

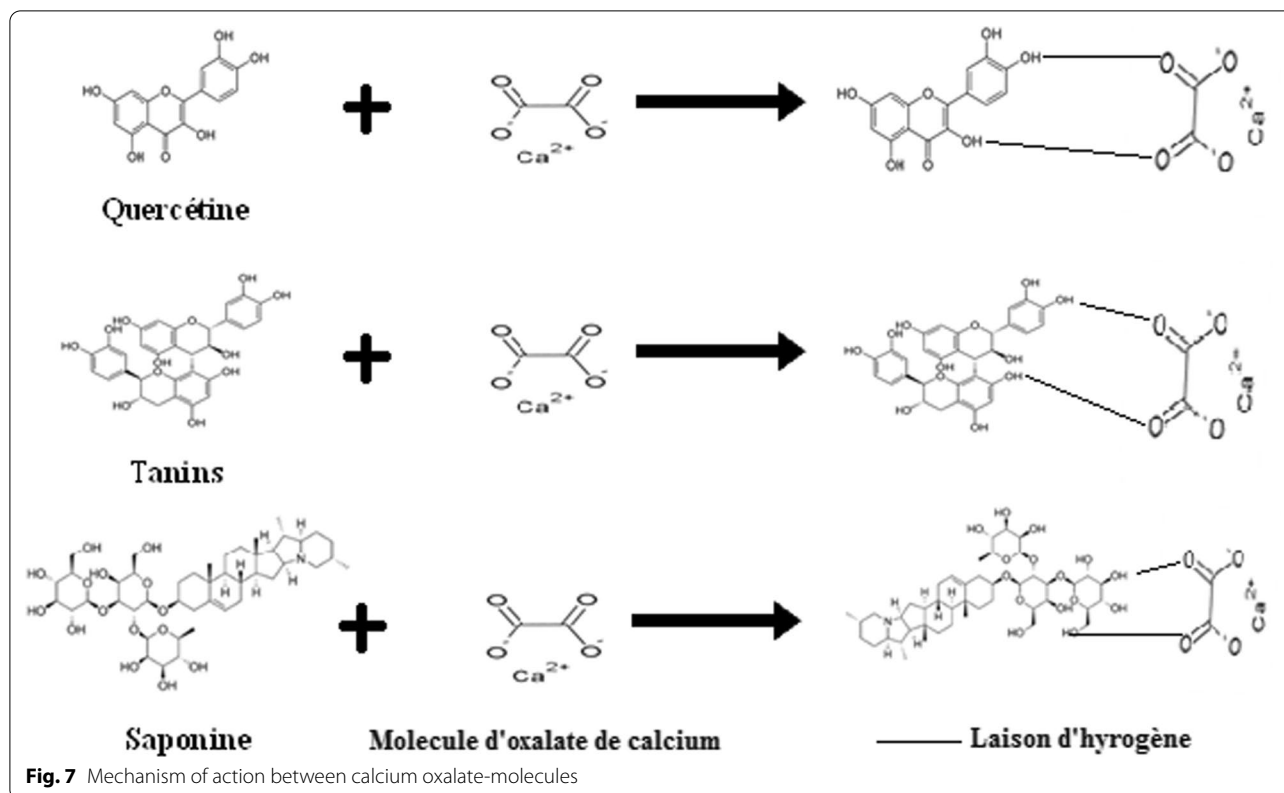
4 Discussion

The crystallization of calcium oxalate is related to the molar calcium oxalate product; however, the crystalline form which is noticed in the urine is strongly dependent on the molar ratio of calcium/oxalate. In this regard, whewellite is oxalo-dependent and formed in urine when the oxalate concentration is above 0.3 mmol/L and the molar calcium/oxalate ration is low [12, 13]. The use of medicinal plants to dissolve whewellite stones remains an interesting alternative.

The tests performed in vitro in the presence of different extracts exhibit a very higher solubilization kinetics than those given by both saline and potassium citrate solutions. In this case, the dissolving power of Whewellite stones shown by extracts of *Z. mays* styles, *Opuntia Ficus-Indica* flowers, *H. hirsuta* L. and seeds of *Ammi visnaga* could be related to the interaction between the calcium oxalate and the molecules present in the plant extracts tested. On the other hand, the results obtained from the four plants are largely exceeding those obtained by Khouchla et al. [14] who worked on the *Zizyphus lotus* L aqueous extract and kachkoul et al. [15] on the infusion and ethanolic extract of *A. unedo* plant.

However, the studies relating to the chemical composition of the four plants show that the *H. hirsuta* L plant





contains saponosides such as medicagenic acid, bidesmosidic saponosides and flavonoids derivatives. *Z. mays* styles are rich in polyphenols, tannins, and potassium, while *Ammi visnaga* L. seeds are mostly composed of furanochromes like khellin, visnagin. As for the flowers of *O. ficus-indica*, we deduce that they are mostly rich in flavonoids, pendulum, rutin, quercetin and luteolin [16–19]. In fact, catechin (flavonoid) shows a preventive effect of the calcium oxalate crystallization induced by ethylene glycol [20]. Also, quercetin and hyperoside have been shown a significant effect in inhibiting the deposition of calcium oxalate crystals in the rats kidneys [21].

The examination of all chemical constituents present in the various plants used suggests that a mechanism of action independent to the pH maybe responsible for the dissolution of whewellite stones. Therefore, this effect could be attributed to the formation of complex oxalate-molecules, such as oxalate-saponin, oxalate-tannins or oxalate-quercetin (Fig. 7) of which the stability would be both ensured by hydrogen and hydrophilic links between the functional groups of active molecules and calcium oxalate. Consequently, the complexes formed are very soluble than calcium oxalate which causes the dissolution of the stones while maintaining in solution the high amounts of dissolved calcium oxalate. Indeed, Meiouet et al. [8] have already proposed almost the same

mechanism to explain the plant extracts effect on cystine stones.

5 Conclusion

The outcomes of our experiments display the efficiency of four plants extracts in the dissolution urinary stones. In this respect, these extracts may have interesting use as curative and/or prophylactic agents for patients with urinary tract stones. However, an optimization study is necessary to determine the optimum concentration of the plant used.

Abbreviations

HHL: *Herniaria hirsuta* L.; OFI: *Opuntia ficus-indica*; ZMS: *Zea mays* styles; AVL: *Ammi visnaga* L.; W: weight; DR: dissolution rate; C pot: potassium citrate; S phy: physiological saline solution.

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Author contributions

RH conceived and designed the experiments; performed the experiments; analyzed and interpreted the data; and wrote the paper. TSH, AL, and AC conceived and designed the experiments; analyzed and interpreted the data. MM, RK, and BAC performed the experiments and analyzed and interpreted the data. All authors read and approved the manuscript."

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

We have not working on the animal model or on the patients.

Consent for publication

Not applicable.

Competing interests

We wish to confirm that there are no known conflicts of interest associated with this publication.

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