In vitro anti-crystallizing activity of the fruit of the Arbutus-Unedo in the human urine
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Abstract
Context: In Morocco, so-called forgotten fruits such as the fruits of the Arbutus Unedo can be valorized within the framework of sustainable development as a local and regional product. The aim of this work is was to evaluate, in vitro, the anti-crystallizing activity of the Arbutus Unedo of choice against calcium oxalate crystals in human urine.

Methods: the fruits of the Arbutus Unedo were collected from the Moulay Issa Ben Driss area of the Beni Mellal-Khenifra region. The aqueous extract of the fruits was prepared in distilled water at a concentration of 10 mg/ml and subjected to cold maceration at 4°C for 48 hours.

The in vitro crystallization of calcium oxalate in human urine was carried out using the Polarized Light Optical Microscope (PLOM).

Results: the addition of the aqueous extract of the grater of the fruit of Arbutus unedo to human urine decreased the size and number of crystals of calcium oxalate di-hydrate and the fruit showed anti-lithiasis activity in vitro by inhibiting the growth of calcium oxalate crystals. Thus, inhibiting the aggregation of calcium oxalate crystals as a lithogenic species.

Keywords: Arbutus Unedo; calcium oxalate; anti-lithiasis activity; human urine

Résumé
Contexte: Au Maroc, il y a des fruits dits oubliés comme dans le cas des fruits de l’Arbutus Unedo peuvent être valorisés dans le cadre du développement durable régional en tant que produit local et produit de terroir

L’objectif du présent travail est d’évaluer, in vitro, l’activité anti-cristallisant du “fruit oublié” de choix vis-à-vis les cristaux de l’oxalate de calcium dans l’urine humaine.

Méthodes: Les fruits de l’Arbutus Unedo ont été prélevés de la zone Moulay Issa Ben Driss de la région Béni Mellal-Khenifra. L’extrait aqueux du fruit de l’étude a été préparé dans l’eau distillée à la concentration de 10mg/ml et soumis à une macération à froid à 4°C pendant 48 heures.

L’étude de la cristallisation, in vitro, de l’oxalate de calcium dans l’urine humaine a été réalisée grâce au microscope optique à lumière polarisée (MOLP)


Mots-clés: Arbutus Unedo, oxalate de calcium, activité anti-lithiasique, urine humaine

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Introduction

The prevalence of urolithiasis is increasing worldwide and calcium oxalate (CaC2O4) is the main component of the majority of urinary stones developed in the urinary tract of lithiasis patients [1]. Calcium oxalate can exist in three crystalline forms more or less hydrated and determined according to the number of the water molecule in the chemical formula. A distinction is made between calcium oxalate monohydrate CaC2O4,1H2O: OCM or Whewellite; calcium oxalates di-hydrate CaC2O4,2H2O: OCD or Weddellite and calcium oxalate trihydrate CaC2O4,3H2O: OCT [2].

Arbutus Unedo (Ericaceae) is among the medicinal plants used to treat cardiovascular disease, diabetes and hypertension. In fact, it would improve renal function [3-5]. Arbutus Unedo could be used as a natural preservative for foodstuffs [6]. Arbutus Unedo is said to be rich in antioxidants: carotenoids such as B-carotene, Violoxanthan and Neoxanthin [7]; polyphenols such as flavanols glycosides and tannins [8]. Arbutus unedo has an in vitro inhibitory effect on the aggregation of human platelets [9, 10]. Its aqueous extract could prevent oxidation and inflammatory processes thanks to the antioxidant activities it possesses [11]. The aqueous extract of Arbutus unedo is said to have antibacterial activity against Escherichia coli and Staphylococcus aureus, thanks to the following constituents: quinones, anthraquinones, anthocyanins, tannins and flavonoids [12]. The medicinal plant Arbutus Unedo has anti-leishmanial activity [13]. Thus, the aim of this work is to investigate the inhibitory effect, in vitro, of aqueous extracts of the fruit of Arbutus Unedo on the crystallization of calcium oxalate di-hydrate (Weddellite) in the urine of patients with urolithiasis.

Materials and methods

1. Collection of plant and extract preparation:

The fruits of Arbutus Unedo were collected in April 2018 from the Moulay Issa Ben Driss area of the Beni Mellal- Khenifra region and stored in the refrigerator. They were grated using the mortar to obtain a rasp used to prepare aqueous extracts (Figure 1). The aqueous extract of the fruits’ Arbutus Unedo was prepared in distilled water at a concentration of 10 mg/ml and subjected to cold maceration at 4°C for 48 hours.

2. Preparation of urine samples:

The collection of urine samples from patients with urinary lithiasis takes place at the multidisciplinary JABRANE Clinic in Beni Mellal. Urine was collected in a clean 250 ml capacity container and stored at room temperature and sent to the faculty’s analytical laboratory within two hours of urination. Upon receipt of the sample, their pH was measured with sufficient accuracy by a pH meter (Figure 2). Using a Pasteur pipette, part of the urine was collected from the bottom of the container and returned to the middle to collect the larger crystals and aggregates and then transferred to a Malassez cell for microscopic examination. The other part of the sample was destined to study the anti-crystallizing activity of calcium oxalate by adding the aqueous extract of the rasp of the fruit of Arbutus Unedo.

![Figure 1: (a): Fruit of the Arbutus Unedo, (b): grater of the fruit of the Arbutus Unedo](image1)

![Figure 2: pH meter devices](image2)
vitro of crystals that would not be of real significance for the established study. Urine should be stored at room temperature or 37°C (but not 4°C) between the time it is released and the time it is examined. The optical microscope must be equipped with a polarization device to carry out a qualitative and quantitative study and for proper crystal identification.

Results and discussions

1. Microscopic urine observation results without extract:

The Hydrogen Potential pH is measured for each urine sample collected. Ideally, urine should be emitted to the laboratory and examined without delay, but these technical constraints make it difficult to carry out this examination on a daily basis. Studies of urine storage at room temperature or 37°C have shown that if the sample was stored less than 3 hours after emission above 20°C, the evolution of crystalluria was very small and the results were interpretable in the same way as those obtained on freshly emitted urine \[14\]. In most cases, several ions, including at least one in excessive concentration in the urine, will combine to form a chemical species such as calcium can form calcium oxalate with the oxalate ions \[15\]. This species is insoluble: their solubility is \(3 \times 10^{-9}\) (mmol/l)\(^2\) for whewellite in aqueous medium \[16,17\]. Crystals are identified by their morphological characteristics and their appearance in polarized light. Crystalline species independent of the PH are few in number. This is the case of calcium oxalate which is partly dependent on PH but over the usual range of urine between 4.8 and 7.5 and their sensitivity can be considered low \[15\]. The crystalline facies are another important element in crystalluria \[15\]. Indeed, not all crystals of a given species necessarily have the same shape \[17,18\]. These depend essentially on two elements: the biochemical composition of the urine and the presence of substances capable of interfering with the growth of certain crystal surfaces. This results in a change in the shape of the observed crystals \[15\]. Calcium oxalate dihydrate: weddellite is in the form of octahedral crystals composed of two flattened pyramids joined by the base and thus appear in the form of square envelopes (Figures 4 and 5). When calciuria rises, an increasing number of weddellite crystals show a thickening of the separating edge between the two pyramids.

In Figure 5 the pH of the urine is 5.7 and the only crystallographic form observed is weddellite and the crystals form an aggregation between them. A study by Daudon shows that in a highly hypercalciuric urine, the only observable facies is the weddellite \[15\]. The characteristic crystals of calcium oxalate monohydrate: whewellite are oval with a depressed center and swollen at the ends (Figure 4), it is essentially associated with hyperoxaluria \[15,20\].

2. Result of crystallization of calcium oxalate in human urine by adding the extract of the fruit Arbutus Unedo:

The inhibitory effect of the aqueous extract of the fruit of Arbutus Unedo on the crystallization of Calcium Oxalate is evaluated in vitro in human urine at a concentration of 10 mg/ml of the Arbutus Unedo.
fruit rasp. Results of previous work carried out by our team [21] have shown that there is reduction of calcium oxalate crystal aggregates in vitro in quantity under the effect of dilution with distilled water. After three tests, we brought to 37°C a volume of 10 ml of the aqueous fruit extract of Arbutus Unedo with a concentration of 10mg/ml and a volume of 10 ml of human urine. After 2 hours of magnetic agitation we observed on an optical microscope with polarized light at a magnification of 200 the development of calcium oxalate crystals in urine with aqueous extracts from the study fruit. Deepti et al [22] have approached the anti-lithiasis effect of aqueous extract from album leaves of Chenopodium album by studying the in vitro crystallization of calcium oxalate. According to the study by Montealegre et al. [23], the dose of 0.5 and 1 mg/ml of Blumea Balsamifera extract will decrease the crystal size of calcium oxalate, also tends to shift the crystal phase to the calcium oxalate dihydrate (COD) phase and inhibits crystal aggregation. Sarsimtha et al [24] worked on aqueous and alcoholic extracts of Bergenia Ciliata rhizome by questioning their inhibitory effect on nucleation and the aggregation of calcium oxalate crystals. Previous work has described a method for isolating methanol-soluble and insoluble fractions from Humulus Lupulus L. rich in compounds that inhibit the formation of calcium oxalate. In fact, both fractions may be effective in the treatment of kidney stone disease [25]. Similarly, the anti-crystallizing effect against calcium oxalate by extract of Dolichos Biflorus seed in vitro has been confirmed [26]. In addition, Magnesium, citrate and phytate are inhibitors of calcium oxalate crystallization, preventing the formation of calcium oxalate monohydrate and trihydrate and preventing calcium oxalate crystallization by decreasing its supersaturation [27]. The study of the Moroccan medicinal plant Zizyphus Lotus brought by our team [21] had shown that 4mg/ml of the aqueous extract of the powder of the Z pulp. Lotus inhibits the aggregation of calcium oxalate in aqueous calcium oxalate solution. Bellakhdar et al [28] studied the effect of Zyziphus Lotus leaves in the treatment of urinary tract infections. Through this work, 10mg/ml of the aqueous extract of the fruit rasp of Arbutus Unedo shows an anti-crystallizing activity against calcium oxalate, there is a remarkable decrease in the size and number of crystals of calcium oxalate di-hydrate (Weddellite) in the human urine of pH=5.3 lithiasis patients (Figure 6).

**Figure 6:** Micrograph of the crystals of calcium oxalate di-hydrate or weddellite in human urine of pH=5.3 Polarized light optical microscopy (Gross=200):
- (a): Without aqueous extract; (b): with 10 mg/ml of the aqueous extract from the rasp of the fruit of Arbutus Unedo

**Conclusion and perspective**

This work had shown that the aqueous extract of the rasp of the fruit of Arbutus Unedo inhibits in vitro the growth of calcium oxalate di-hydrate crystals in human urine. The anti-crystallizing activity of the aqueous extract of the rasp of the fruit of Arbutus Unedo against calcium oxalate di-hydrate could be due to the presence of phytochemical compounds, further characterization and isolation of the main active component from the fruit of Arbutus Unedo. The in vivo study and clinical trials remain decisive and decisive in the question of the therapeutic and medicinal valorization of the bioactive elements extracted from the medicinal plant Arbutus Unedo. In addition, thanks to this work, the Polarized Light Optical Microscope (POLM), which is a priori a routine technique in terms of laboratory analysis, could be optimized and valorized for the evaluation of urinary lithogenesis.
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Références