




Original Article

Investigation of the potential anti-urolithiatic activity of *Alhagi maurorum* (Boiss.) grown wild in Al-Ahsa (Eastern Province), Saudi Arabia

Investigação da potencial atividade antiurolítica de *Alhagi maurorum* (Boiss.) cultivado selvagem em Al-Ahsa (Província Oriental), Arábia Saudita

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Abstract

The potential of *Alhagi maurorum* (Boiss.) aqueous extract (AME), used in traditional medicine for treatment or prevention of urolithiasis, to dissolve calcium oxalate stones *in vitro* was evaluated. In order to determine the litholytic potential of the extract, Calcium oxalate urinary stones were incubated during 12 weeks under continuous shaking in the presence of AME, Rowanix or NaCl 9 g/mL solution were used as controls. After the incubation period, the residual weight of the treated calculi was determined and the rate of dissolution was calculated. The medium pH variation was measured and changes in the calcium oxalate crystals at the stone surface were assessed using a scanning electron microscope (SEM). The results showed a significant dissolution effect for the extract on the kidney calculi during the experimentation period. At the end of the experiment, the percentages of calculi weight decrease were 41.23, 4.97 and 55.67% for the extract, NaCl solution and Rowanix, respectively. Gas Chromatography analysis revealed mainly the presence of the following phyto-compounds: Cyclopropanone, 2,3-diphenyl; 1-Nonadecanol; methyl-alpha-D-mannopyranoside; cis-9-Hexadecenal. These compounds unarguably play crucial roles in the health care system especially in cancer treatment and many other diseases including urolithiasis. The urinary stone dissolution, independent of medium pH, could be attributed to formation of complexes between the phytochemical compounds in the extract and the calculi.

Keywords: *Alhagi maurorum* (Boiss.), urolithiasis, GC-MS, calcium oxalate urinary stones, pH, scanning electron microscope.

Resumo

Foi avaliado o potencial do extrato aquoso (AME) de *Alhagi maurorum* (Boiss.), usado na medicina tradicional para tratamento ou prevenção de urolitíase, para dissolver cálculos de oxalato de cálcio *in vitro*. Para determinar o potencial litolítico do extrato, cálculos urinários de oxalato de cálcio foram incubados durante 12 semanas sob agitação contínua na presença de AME, Rowanix ou solução de NaCl 9 g/mL foram usados como controles. Após o período de incubação, o peso residual dos cálculos tratados foi determinado e a taxa de dissolução foi calculada. A variação do pH médio foi medida e as alterações nos cristais de oxalato de cálcio na superfície da pedra foram avaliadas usando um microscópio eletrônico de varredura (MEV). Os resultados mostraram um efeito de dissolução significativo para o extrato sobre os cálculos renais durante o período de experimentação. Ao final do experimento, os percentuais de diminuição do peso dos cálculos foram de 41,23, 4,97 e 55,67% para o extrato, solução de NaCl e Rowanix, respectivamente. A análise por cromatografia gasosa revelou principalmente a presença dos seguintes fitocompostos: Ciclopropanona, 2,3-difenil; 1-Nonadecanol; metil-alfa-D-manopiranosídeo; cis-9-hexadecenal. Esses compostos indiscutivelmente desempenham papéis cruciais no sistema de saúde, especialmente no tratamento do câncer e de muitas outras doenças, incluindo urolitíase. A dissolução do cálculo urinário, independente do pH médio, pode ser atribuída à formação de complexos entre os compostos fitoquímicos do extrato e os cálculos.

Palavras-chave: *Alhagi maurorum* (Boiss.), urolitíase, GC-MS, cálculos urinários de oxalato de cálcio, pH, microscópio eletrônico de varredura.

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1. Introduction

Urolithiasis is defined as the formation of stones within the urinary tract (Kaleeswaran et al., 2018). It is a well-known disorder that results from integrated biochemical, epidemiological and genetic risk factors (Makasana et al., 2014). Increase in excretion of stone-forming compounds (e.g., calcium, cystine, oxalate, phosphate, urate and xanthine) or decrease in urine volume is linked with stone formation (Yachi et al., 2018) and Calcium oxalate stones are the common type of nephrolithiasis (Assadi and Moghtaderi, 2017). Several factors have increased the prevalence of urolithiasis: Low- intake of vegetables, fruits and fluids, and high consumption of animal proteins, salt and sweetened beverages contribute significantly in prevalence of the urolithiasis (Yachi et al., 2018). Urinary stone formation happens in roughly 12% of the populace with a repeat rate of 70-80% in man and 47- 60% in females (Kaleeswaran et al., 2018). It can likewise forever harm the kidneys, if not treated appropriately (Chandrajith et al., 2019).

On the other hand, therapeutic plants have a long history of utilization and are universally more secure than manufactured medications (Bahmani et al., 2016). *Alhagi*, is a widespread plant genus that belongs to the family of *Fabaceae*. It can be found in many countries in Africa, Asia, Australia and Europe. The common name of *Alhagi* is camel thorn. *Alhagi* has numerous species with many popular therapeutic uses. Diverse types of *Alhagi* have been investigated for their cell reinforcement potential and nutritive incentive alongside different therapeutic properties (Ekor, 2014). It has been reported that different plant parts of *Alhagi* have a wide range beneficial medical applications in combating diseases such as ulcer, diarrhea and liver diseases. Additionally, *Alhagi* parts exhibited antimicrobial and anti-oxidant activities (Dias et al., 2012; Saki et al., 2014; Ekor, 2014). Furthermore, *A. maurorum* can be used in folk medicine, in treating digestive, urinary and liver diseases (Al-Douri and Al-Essa, 2010). The leaves and flowers of *A. maurorum* are effective in curing disease such as rheumatism, migraine and piles. Water extracts of this plant were reported to help in removing kidney stones and enlarging ureter (Marashdah et al., 2006; Marashdah and Al-Hazimi, 2010; Ahmad et al., 2015).

The aim of this study was to evaluate the dissolving effect of *A. maurorum* aqueous extract to treat renal lithiasis. To this end, we have studied its effect on the *in vitro* variation of Calcium oxalate calculi weight; kinetic aspects of this dissolution, the medium pH variation, the extract chemical profile and calculi structural changes using a scanning electron microscopy.

2. Materials and Methods

2.1. Collection of plant material

The whole plant material of *A. maurorum* was collected in spring from around Hofuf city (Eastern Province, Saudi Arabia). It was authenticated by Dr. Rashid Ismail, Botanist at College of Science, King Faisal University and the plant name was checked with www.theplantlist.org at 17-3-2019.

A specimen, voucher #BSD-AM025-18 was deposited at the Department of Biological Sciences, KFU. Thereafter, the plant material was washed and shade dried at room temperature.

2.2. Preparation of plant extract

The powdered leaves weighing 10 g was soaked in 100 mL of physiological solution (9 g NaCl/L distilled water, pH 6.7) and heated at 80 °C for 30 min. The NaCl solution of 9 g/L being used as control medium to appreciate the weight modifications and/or calculi structure. The hot extract was cooled to room temperature and filtered through Whatman No. 1 filter paper, and the supernatant was collected and stored at 4 °C until further use (Siddiqui et al., 2018).

1g of the powdered extract was extracted by 10 mL of 99.8% Ethanol purchased from Sigma Aldrich. A 250 W ultrasonic processor was used for extraction at 60 °C for 30 minutes. The extract was filtered carefully and 2 mL were transferred to a vial and capped for GC-MS analysis.

2.3. GC-MS analysis conditions

The Extract was monitored and analyzed by Shimadzu gas chromatograph mass spectrometer (GC-MS) model QP2010 SE equipped with Rxi-5 Sil MS capillary column (30 m length, 0.25 mm ID and 0.25 µm film thickness). The analysis was performed using the gas GC parameters as follows: Injector temperature, 250 °C; initial oven temperature, 40 °C (held for 1 min), increased to 300 °C at a rate of 10 °C min⁻¹ and held for 15 minutes. The total time required for GC run was 42 minutes. The inlet was operated in the splitless mode. The MS transfer line was held at 200 °C. The carrier gas is high-purity Helium with a flow rate 1 mL min⁻¹. LabSolution software was used to control the system and to acquire the analytical data. Peak identification was made by comparing the mass spectra of detected compounds with NIST mass spectral library (Hema et al., 2011).

2.4. Urinary calculi

Renal calculi of Calcium oxalate were obtained from the Fattouma Bourguiba University Hospital, Monastir, Tunisia (Urology Department). The calculi have been characterized and selected after a spectrophotometry analysis using optical microscopy and Fourier Transformed Infrared (FTIR) following the method outlined earlier (Benramdane et al., 2008; Yachi et al., 2018).

2.5. Litholytic activity

The *A. maurorum* aqueous extract (AME) was partitioned (60 mL) into glass Erlenmeyer flasks containing the calculi. The flasks were maintained at an ambient temperature, under constant shaking (Shaker type GFL 3020, Burgwedel, Germany) without magnetic stirring. Thus, kidney stones have been isolated from any mechanical effect.

For each experiment, the pH of the solution was measured every 2 weeks using a pHmeter (Jenway 3510, Staffordshire, UK). The weight reduction of calculi was evaluated every 2 weeks by weighing the calculus after drying in an oven at 40 °C for 6 hours. Every experience

was performed in triplicate. Rowanix drug has been used as positive control.

The litholytic potential of the extract has been evaluated by calculating the rate of calculi dissolution by comparing their residual weight with their initial one before the incubation in the presence of the extract.

Calculation of the percentage of calculi dissolution was performed as described earlier (Saso et al., 1998) using the following Equation 1:

$$DP (\%) = \frac{(W_{\text{initial}} - W_{\text{final}}) \times 100}{W_{\text{initial}}} \quad (1)$$

where DP(%): was the dissolution percentage of the calculi; W initial was weight of the calculus at the beginning of incubation; and W final was the weight of the calculus at the end of incubation period.

2.6. Examination of the calculi using Scanning Electron Microscope

A field effect scanning electron microscope (SEM) (JEOL, JSM 7600F, Tokyo, Japan) was used for microstructure observations. To preserve the integrity of the samples, the measurements were made with a GB (gentle beam) mode at a low voltage (1.2 KV) and without any carbon deposits on the surface of the sample.

2.7. Statistical analysis

Data were obtained from three independent experiments and presented as the mean \pm standard deviation (SD). Analysis for statistical significance from control was carried out using the Dunnett test (SPSS 11.5 Statistics Software; SPSS, Chicago, IL). The significance level was taken at $p < 0.05$.

3. Results

3.1. Litholytic activity of AME

The changes in the weight of calcium oxalate urinary stones during 12-week incubation with the extract, the control solution of NaCl and the positive control (Rowanix) are presented in Figure 1A. At the end of the experimentation, the average weight reductions of calculi treated with AME, Rowanix, and NaCl solution were 181 ± 12 mg, 162 ± 23 mg and 15 ± 3 mg, respectively.

The dissolution kinetics seem to be different. During the first four weeks, the decrease of calculi weight varies slightly for the three treatments. Between six and eight weeks, the dissolution rate remained low and began to increase after ten weeks, especially for AME and positive control. The results were confirmed at the end of the experiment, with a high effect on the dissolution of stones, expressed by a significant decrease of weight compared to the control (NaCl solution) (Figure 1B). This calculi dissolution percentage (41.23% for AME vs 4.68% for the control NaCl solution and 55.67% for Rowanix) reached the threshold of significance ($p = 0.05$) over the last four weeks.

3.2. pH variation of the medium

The pH variations of the medium during the experiment are shown in Figure 1C. The initial value ranged from 6.7 for control NaCl solution to 7.31 for AME ($p < 0.05$). We observed a decrease in pH during the first two weeks of the experiment for AME and Rowanix as well as for the control solution. Then the pH decreased slightly and remains between 5 and 6 during the experiment period.

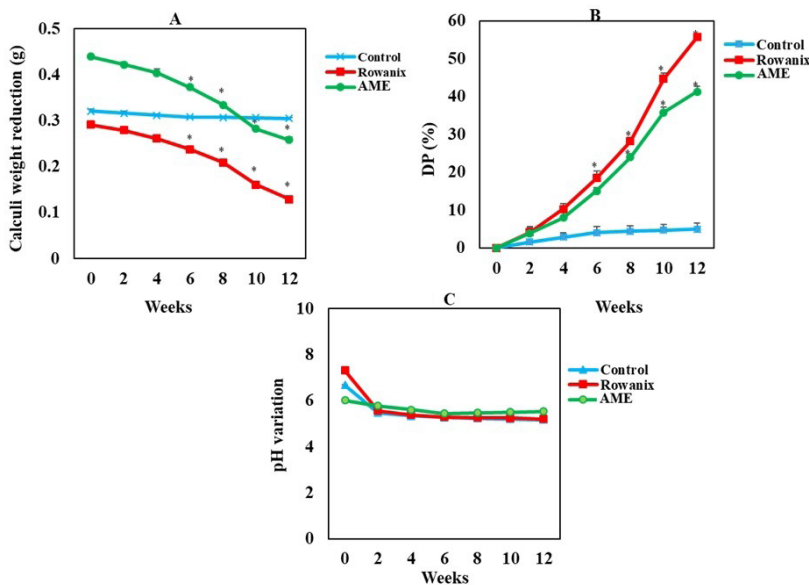


Figure 1. Effect of *A. maurorum* extract on weight decrease of the calcium oxalate calculi (A), kinetics of weight decrease (B), and pH variation (C). *Significant value at $p < 0.05$.

3.3. Scanning Electronic Microscope analysis of calculi

Figures 2A and 2B represent the SEM images of the calcium oxalate crystallites respectively before and at the final stage in the case of the NaCl solution compared to those of calculi incubated with the AME (Figure 2C). Although differences seem to exist at the microscopic scale between the surfaces of the stones subjected to the action of plant extract compared to that observed before treatment, it is difficult to quantify them. The treatment appears to have partly dissolved the small calcium oxalate crystals, the largest ones of them are visible and separated by more or less wide voids. The NaCl solution seems to have had little effect on the structure of stones (Figure 2A). The Rowanix (Figure 2D) has an effect more clear as compared to that of AME. The organization of the crystals appear laminated due to a different orientation.

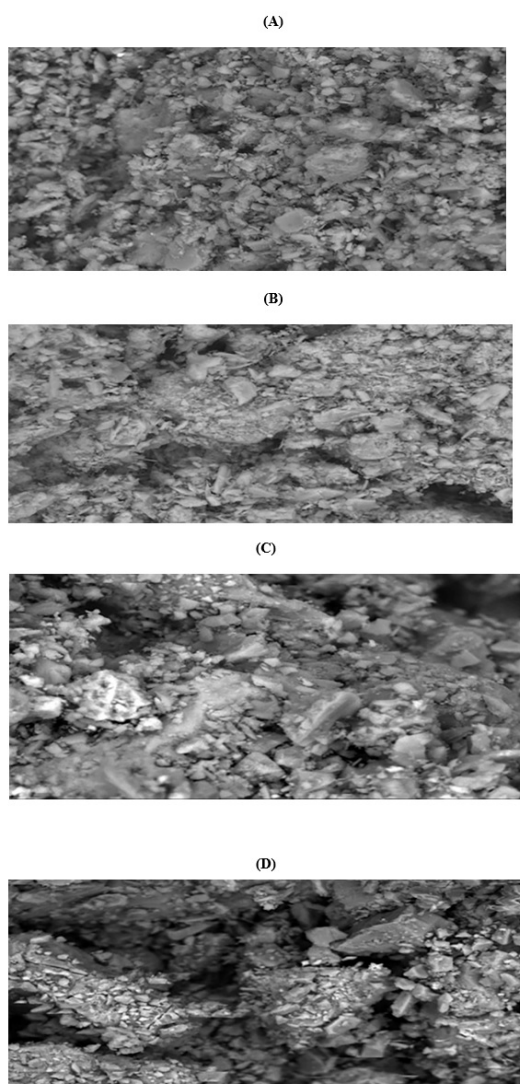


Figure 2. Crystal surface visualized by SEM: (A) before any treatment; (B) after treatment with the control (NaCl solution, 9 g/L); (C) after treatment with the solution containing Rowanix; (D) after treatment with the solution containing AME.

3.4. GC-MS analysis of AME

GC-MS analysis of AME revealed the presence of six major bioactive compounds as shown in Table 1 and Figure 3. From the results of GC-MS spectra, Cyclopropanone, 2,3-diphenyl (Aromatic compound), 1-Nonadecanol (Alcohol), methyl α -D-mannopyranoside and cis-9-Hexadecenal (Aliphatic compound) are the most abundant while 1-Undecanol, 1-Tetradecene and 2,4-Di-tert-butylphenol are the least. These bioactive compounds has been reported to play important roles in diseases and general metabolism of humans (Maretti et al., 2017; Awad et al., 2018; Ngomback et al., 2019).

4. Discussion

Several medicinal plant species have been investigated for their effect in treating urolithiasis both in *in vitro* and in *vivo* (Chilivery et al., 2016; Abu Zarin et al., 2020; Mosquera et al., 2020). The plant selected for this study is widely used for the treatment of renal calculi or to prevent their formation (Badshah and Hussain, 2011; Mikaili et al., 2012; Muhammad et al., 2015; Varshochi and Asadollahi, 2015).

Calcium oxalate is the most common crystalline form. Indeed, Among the four major types of stones, calcium, uric acid, struvite and cysteine, the calcium oxalate accounts for more than 80% of reported cases (Basavaraj et al., 2007), and this type of calculi is often difficult to treat and to prevent by the urologic and medical procedures.

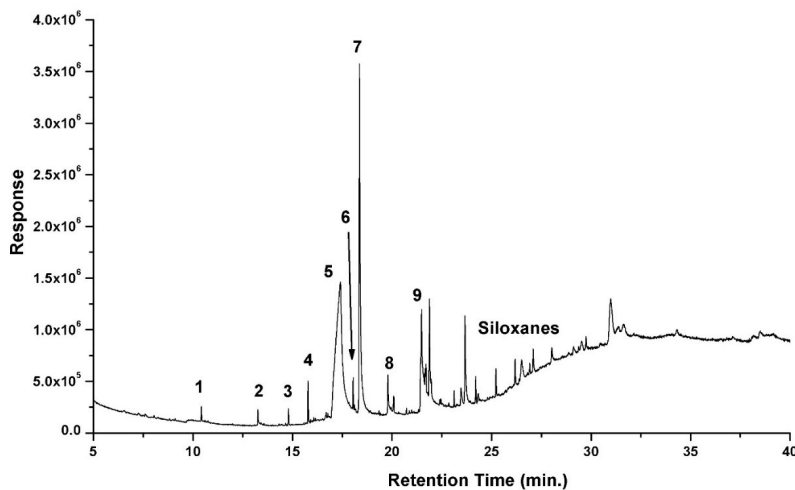
In this work, we have investigated the *in vitro* effect of *A. maurorum* aqueous extract to dissolve calcium oxalate renal stones and we have evaluated the effect of the tested extract as well as control by calculating the rate of calculi dissolution after a period of time by comparing the residual weight of calculi with their initial weight.

The results have shown a significant dissolution effect for AME for the tested kidney calculi during the twelve weeks period of experimentation. However, the examination of Figure 1B shows that the the dissolution curve obtained for AME increased sharply in the last four weeks compared to the curve observed with the NaCl solution and even with those of Rowanix, suggesting that this plant is probably effective in dissolving calcium oxalate stones, but probably requires more time. It has been shown that plants used in traditional medicine in Morocco against kidney stones in general were able to effectively dissolve stones *in vitro* (Meiouet et al., 2011; Hannache et al., 2012). Also, we studied the microscopic features of calculi using field-effect SEM to follow the possible changes in the morphology of the crystallites, searching for eventual interactions between plant extracts and calculi. Numerous studies based on SEM observations of renal calculi (Dorian et al., 1996; Abdel-Aal et al., 2009) have largely demonstrated that this technique was well adapted to this domain. With SEM, it was possible to observe the details of the surface of the crystallites without having to cover the sample with a conductive layer of carbon. This makes it possible to repeat the observations during the experiment without modifying the surface of the samples by a treatment,

Table 1. Phytochemical components of AME extract using GC-MS.

S/no	RT (min)	Name of compound	MW (g/mol)	MF
1	10.42	1-Undecanol	172	C ₁₁ H ₂₄ O
2	13.25	1-Tetradecene	196	C ₁₄ H ₂₈
3	14.79	2,4-Di-tert-butylphenol	206	C ₁₄ H ₂₂ O
4	15.78	1-Pentadecanol	228	C ₁₅ H ₃₂ O
5	17.42	methyl α -D-mannopyranoside	194	C ₇ H ₁₄ O ₆
6	18.04	1-Nonadecanol	284	C ₁₉ H ₄₀ O
7	18.36	Cyclopropenone, 2,3-diphenyl	206	C ₁₅ H ₁₀ O
8	19.8	Pentadecanoic acid	242	C ₁₅ H ₃₀ O ₂
9	21.47	cis-9-Hexadecenal	238	C ₁₆ H ₃₀ O

RT = retention time; MF = molecular formula; MW = molecular weight.

**Figure 3.** GC-MS profile of AME leaf extract.

which could generate the subsequent action of the plant extracts tested.

Regarding the medium pH, the initial value appears slightly different according to the extracts, between 6 and 7.3. The low pH value suggests an acidification effect on the dissolution of calcium oxalate stones by the extracts. However, the comparison of the pH curve obtained with the NaCl solution to that obtained with the Rowanix positive control or the AME revealed that the curves are very similar, suggesting that the effects of plant extract on the dissolution of stones are not related to the pH but to the chemical composition of the plant extract. Besides, previous phytochemical studies have shown a wide range of pharmacologically active compounds such as alkaloids, flavonoids and polyphenols that were identified in different parts of *A. maurorum* (Muhammad et al., 2015; Ahmad et al., 2015; Suthar et al., 2016). Such compounds could form complexes with the calculus “calculus-active principle complex” rendering it more soluble than calculus itself.

5. Conclusion

The presented data indicated that *A. maurorum* leaf extract possesses anti-urolithiatic properties by its dissolving effect on Calcium oxalate calculi, kinetic aspects of this dissolution and calculi structural changes using a scanning electron microscopy. The results provided experimental evidence for the *in vitro* potential anti-urolithiatic effect of *A. maurorum* leaf extract in kidney Calcium oxalate calculi. These results could be attributed to the formation of complexes between the phytochemical compounds of the extract and the calculi. Therefore, it can be concluded that *Alhagi maurorum* is effective in *in vitro* kidney stone dissolution and could be used after further investigations in preventing urinary stone formation. These data might give additional insight to urologists to design new phytotherapy regimen urolithiasis. It can also help in further *in-vitro* and *in vivo* research to identify bioactive molecules with anti-urolithiatic activity from *A. maurorum*, clarify mechanisms of action and develop natural urolithiasis inhibitors.

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