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Original Research Article

Evaluation of the Acute Diuretic Activity and Acute Toxicity of Hydroethanol Extract of the Fruits of *Ammodaucus leucotrichus*

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ABSTRACT

Ammodaucus leucotrichus, an important plant in Moroccan traditional medicine, is used to relieve various symptoms and pathologies, such as hypertension. This study aimed to evaluate the diuretic and acute toxicity effects of the hydroethanol extract of *Ammodaucus leucotrichus* fruits (ALFE). ALFE was prepared by cold maceration in 75% ethanol and 25% water. Healthy Wistar rats, preloaded with isotonic saline (0.9% NaCl, 25 mL/kg), were divided into five groups of six rats each: Negative control (distilled water 5 mL/kg; orally), Positive control (furosemide 13 mg/kg; orally), and three groups treated with ALFE at doses of 100, 200, and 300 mg/kg, respectively as single oral dose. Acute oral toxicity was assessed in adult albino mice at a single oral dose of 2000 mg/kg, Urine volume and electrolyte (Na^+, K^+, Cl^-) levels were measured 24 hours after treatment. All doses significantly increased urine output ($P < 0.001$) at 1, 2, 4, 6, and 24 hours compared to the negative control. The highest ALFE dose (300 mg/kg) caused pronounced and sustained polyuria comparable to furosemide at 24 hours. Electrolyte excretion also increased significantly ($P < 0.001$) across all doses compared to the negative control and furosemide. Acute oral toxicity test showed no mortality or sign of toxicity over 14 days observation period. Furthermore, no significant weight loss or behavioral abnormalities were observed, indicating that the extract exhibits low acute toxicity at the tested dose. These findings confirm the potent diuretic effect of ALFE and support its traditional medicinal use for promoting diuresis.

Keywords: *Ammodaucus leucotrichus*, Diuretic Activity, Electrolyte Excretion, Acute Toxicity, Rats

Introduction

Natural resources, particularly plant species have been exploited since ancient times for numerous purposes, including nutritional, therapeutic, or cosmetic applications through the use of plant part or whole plant.[1] Medicinal and aromatic plants contain secondary metabolites, bioactive substances, which present a wide range of potential biological properties. These properties serve as a scientific foundation for the integration of plants into traditional medicine.[2] Approximately 64% of people around the world use plant species to prepare various therapeutic remedies, employing different preparatory methods such as maceration, decoction, or infusion.[3] In the United States for example, about 24% of citizens use medicinal plants to treat certain diseases.[4] In Japan, the demand for plant-based medicines is on the increase.[5]

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Morocco is particularly characterized by a rich and varied flora, with a significant number of endemic plants. Morocco has a large reservoir of plants, accounting for nearly 42,000 species, of which about 600 species and subspecies are used as traditional remedies. The country is also well-grounded on the use of medicinal plants.[6] *Ammodaucus leucotrichus* Coss & Dur (*Cuminum maroccanum* P.H. Davis & Hedge) is the only species of the genus *Ammodaucus* in the Apiaceae family, characterized by its small size and fruits in the form of long diachenes of 6-10 mm covered with dense, silky hairs. Moroccans call it "Kamune es-sofi," also known among the population of southern Morocco as "Kammûn er rag".[7,8] The plant is classified among rare and threatened species. Its preferred habitat is the desert, often growing on sandy coasts and at the foot of a hill or dune, and exists in North Africa.[8] This annual glabrous plant has pointed stems branched at the base. The leaves are fleshy, finely divided, with narrow, flat veins, and sheathing petioles. The white flowers, with five free petals, are arranged in umbels of two to four petals. The fruit is a diakene 8 to 10 mm long, covered with very dense, soft hairs (Figure 1).[8,9] *Ammodaucus leucotrichus Coss & Dur* occupies a crucial place in traditional medicine, it is used in the treatment of several diseases, including pain and gastrointestinal disorders such as vomiting, nausea, regurgitation, gastralgia, indigestion, as well as heart diseases, otitis, chills, dehydration, fevers, lung diseases, childbirth pains, anorexia, and hypersecretion of urine.[7,10,11] Scientific studies have highlighted several biological and pharmacological activities of *Ammodaucus leucotrichus* fruits, notably its protective role against urinary lithiasis.[12] Additionally, essential oils extracted from *Ammodaucus leucotrichus* have revealed strong

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antimicrobial activity and antioxidant properties,[13] as well as antidiabetic effects.[14] This plant species was selected due to its diuretic and hypotensive properties reported in several ethnobotanical surveys.[15,16] Although *Ammaudocus leucotrichus* is traditionally considered a potentially toxic plant,[17] no scientific study has, to date, evaluated the acute toxicity of its extracts. Only one study has reported the toxic effects of its essential oil.[10] To our knowledge, no prior study has investigated the diuretic effect and acute toxicity of *A. leucotrichus* extract samples. This present study aims to contribute to the valorization of this species by examining, for the first time, the diuretic activity as well as the acute toxicity profile of the hydroethanol extract of the fruits of *A. leucotrichus* using an *in vivo* experimental model in rats and mice.

Figure 1: *Ammodaucus leucotrichus* plant in its natural habitat

Materials and Methods

Collection and identification of plant material

Ammodaucus leucotrichus Coss. & Dur whole plant was collected in March 2023 from M'Hamid El Ghizlane, in the Oued Amrir region, a village located 120 km south of the city of Zagora, in southwestern Morocco (GPS coordinates: 29°49'56"W). Based on ethnopharmacological data and traditional uses, the plant material was identified and authenticated by Professor Hamid Khamar, a botanist at the Scientific Institute of Rabat, Morocco. A herbarium specimen was deposited with the voucher number: RAB114865 in the herbarium of the Scientific Institute of Rabat.

Extract preparation

The plant material (fruits) was separated from the aerial part. Subsequently, they were carefully dried in the shade at room temperature for 20 days. After drying, the fruits were reduced to powder using an electric grinder (Vevor Grain Grinder, HC 300, China). A quantity of the powdered sample (50 g) was extracted with 1 L of cyclohexane in a Soxhlet extractor to defat the plant material. After drying in an oven at 40°C for 24 hours, the marc was extracted again by cold maceration with a mixture of 75% ethanol (750 mL) and 25% water (250 mL) at room temperature, with daily agitation for 3 consecutive days.[18] The mixture was filtered using Whatman filter paper. Thereafter, the organic solvent was evaporated from the extract under reduced pressure using a rotary evaporator (Rotavapor R-200, Buchi, Switzerland) at 40°C and 50 mm Hg. The resulting extract was further dried by lyophilization using a lyophilizer (Free Zone® Dry 4.5, USA). The final extract was a yellow powder, which was stored in a refrigerator at 4°C until needed for the experiment.

Preparation of reference drug (furosemide)

Furosemide 40 mg tablets (Lasilix, Pharma 5, Morocco), a high-ceiling loop diuretic, was used as the reference drug (positive control). It was dissolved in distilled water prior to administration.

Pharmacological studies

Experimental animals

Healthy albino Wistar rats (both sexes, 6 to 8 weeks old, weighing 170 – 220 g) and Swiss mice (weighing 21 – 36 g) were used in this study. The rats were obtained from the animal facility of the Biology Department, Faculty of Sciences, Mohammed V University, Rabat, Morocco, while the mice were obtained from the central animal facility of the Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco. All animals were housed in appropriate and comfortable polypropylene cages, with six animals per cage. Before the start of the experiments, the animals were acclimatized to the environment and standard laboratory conditions for about 10 days, including a temperature of 25 ± 2°C and a 12-hour light/dark cycle. The animals were fed a standard diet based on barley pellets, with unlimited access to drinking water.

Ethical consideration

The experiments were conducted in accordance with recognized international guidelines.[19] Ethical approval was granted by the research ethics committee, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat 10100, Morocco.

Evaluation of diuretic activity

Experimental design

The diuretic activity experiment was performed using the method employed by Kau *et al*. (1984)[20] with a slight modification, to identify the diuretic activity of the plant extract. First, the rats, both male and female, were randomly divided into five groups, each group comprising six rats (N = 6). Before administering the treatment, all animals were fasted overnight for 18 hours, while having free access to drinking water during this period. Then, all rats were weighed before being preloaded with isotonic saline solution (0.9% NaCl) at 25 mL/kg,[3] to impose a uniform hydric and saline load. The rest of the experiment then proceeded as follows:

Group 1: (Negative control) the animals were administered orally 5 mL/kg (bw) of distilled water (dw), serving as a vehicle.

Group 2: (Positive control) the rats received, by gavage, the reference drug furosemide at a dose of 13 mg/kg (Fr13), dissolved in 5 mL of distilled water.

Groups 3, 4, and 5: The animals in these groups were treated orally once with the extract of *Ammodaucus leucotrichus* fruits (ALFE) at doses of 100, 200, and 300 mg/kg of (bw), respectively, each extract being dissolved in 5 mL/kg of distilled water.

After the administration of treatments, each rat was placed in an individual standard metabolic cage, designed to separate urine and excrement. Cumulative urine output was recorded at 1, 2, 4, 6, and 24 h after dosing, and was used to determine the urinary volumetric excretion (UVE). During this period, all rats were maintained on fasting, without access to food or water.

Measurement of urine volume

The animals were carefully monitored during urine collection, with cumulative urine volume measured and recorded at intervals of 1 h, 2 h, 4 h, 6 h, and 24 h.

Determination of urine pH

The pH was directly determined on fresh urine samples from all groups after 24 h, measured using a calibrated digital pH meter.

Determination of diuretic action and activity

To evaluate the diuretic effects of three different doses of ALFE compared to negative and positive controls, parameters such as the volumetric urinary excretory rate (UVE) (Formula 1), diuretic action (Formula 2), and diuretic activity (Formula 3) were calculated using the formulas below, considering the average urine volume expressed in 100 mL/g of body weight (bw).[21]

$$\text{UVE} = \frac{\text{volume of urine excreted (expressed as mL/100 g)}}{\text{Total liquid administered}} \times 100 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

$$\text{Diuretic action} = \frac{\text{Urinary exretion output}}{\text{Total excretion of control group}} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of test drug}}{\text{Diuretic action of standard drug}} \quad \text{..... (3)}$$

Determination of saluretic, natriuretic, and carbonic anhydrase inhibitory activity

The concentrations of electrolytes; sodium, potassium and chloride ion (Na^+, K^+ and Cl^-) were determined using a high-precision flame photometer (FP8400 Flame Photometer, A.KRUSS OPTRONIC GmbH, Germany). In addition, the ratios Na^+/K^+ and $Cl^-/ [K^+ + Na^+]$ of electrolytes (test/control) were calculated to evaluate the saluretic, natriuretic, and carbonic anhydrase inhibitory activity of the hydroethanol extract of the plant.

Acute toxicity study

The evaluation of the toxicity of a compound with therapeutic potential represents an essential step in the process of developing new drugs in pharmaceutical research. The acute toxicity of the hydroethanol extract of *Ammodaucus leucotrichus* fruits was evaluated in 6 adult female mice [3 for hydroethanol extract and 3 for control (untreated group)], whose weight varied between 21 and 36 g. The experimentation was conducted in accordance with the guidelines of the Organization for Economic Co-operation and Development (OECD 423). The tests were conducted in accordance with internationally recognized recommendations for the evaluation of the safety and efficacy of phytotherapeutic preparations. Four hours before the administration of the extract, the animals were fasted, while maintaining free access to water. Each mouse was then isolated in a sterile polypropylene cage, and the extract was administered orally using an esophageal probe, at a single dose of 2000 mg/kg, in accordance with the recommendations of OECD guide for substances with potential moderate to low toxicity. After administration, the animals were observed during the first 30 minutes, then daily for a period of 14 days. The evaluated parameters included variations in body weight, mortality, as well as the appearance of clinical signs of toxicity (convulsions, salivation, diarrhea, lethargy, drowsiness, coma, etc.). All observations were systematically recorded.

Gas chromatography-Mass spectrometry analysis

Gas chromatography–mass spectrometry (GC-MS) analysis was performed using a Clarus 580 gas chromatograph (Perkin Elmer) coupled to a Clarus SQ8S mass spectrometer. The system was equipped with a 5MS capillary column (30 m × 0.25 mm × 0.25 µm). A 0.5 µL sample of ALFE was injected in split mode, with helium as the carrier gas at a flow rate of 1 mL/min. The injection temperature was set at 250°C. The oven temperature program started at 50°C, increased at a rate of 10°C/min to 200°C with a hold time of 25 minutes, then increased again to 230°C at 10°C/min with a final hold time of 10 minutes, resulting in a total run time of 53 minutes. The transfer line and ion source temperatures were maintained at 250°C. Data acquisition was performed in EI+ mode with a full scan range of 10 – 600 m/z. Compound identification was carried out by comparing the obtained mass spectra with reference spectra from the NIST library.

Statistical analyses

The data obtained were analyzed using GraphPad Prism (v8.0.2). Results were presented as mean values ± standard error of the mean (SEM). Statistical differences between groups were evaluated using two-way ANOVA, followed by Bonferroni's post hoc test for multiple comparisons. A P-value of less than 0.05 was considered statistically significant.

Results and Discussion

Diuretic activity of ALFE

In this experiment, the diuretic effect of the hydroethanol extract of AL fruits, a North African plant commonly included in plant-based therapeutic remedies,[15] was evaluated in normal adult male and female Wistar rats and compared to that of the negative control and furosemide as a standard drug. The different doses of ALFE (100, 200, and 300 mg/kg) presented the following values for the diuretic index: 1.10, 1.54, and 2.11, respectively, compared to 2.09 for furosemide 13 mg/kg (Table 1). Moreover, the values obtained for diuretic activity were as follows: 0.53 for the dose of 100 mg/kg; 0.74 for the dose of 200 mg/kg, and 1.01 for the dose of 300 mg/kg (Table 1). These two parameters were identified from urine collected at the end of the experiment (24 hours) from different groups of animals.

Table 1: Effects of oral administration of *Ammodaucus leucotrichus* fruits extract and Furosemide on urinary excretion, diuretic index and diuretic activity

Group	Urine volume (mL/100 g)					Urinary volumetric excretion (%)					Diuretic index	Diuretic activity
	1 h	2 h	4 h	6 h	24 h	1 h	2 h	4 h	6 h	24 h	24 h	
DW	0.80 ± 0.40	1.00 ± 0.49	1.23 ± 0.54	1.53 ± 0.55	2.15 ± 0.84	32.0 ± 16.0	40.0 ± 19.6	49.2 ± 21.6	61.2 ± 22.0	86.0 ± 33.6	1.00	
Fr13	3.08 ± 0.57^{a3}	3.50 ± 0.60$^{a\ 3}$	4.06 ± 0.70^{a3}	4.11 ± 0.62^{a3}	4.50 ± 0.70^{a1}	123.2 ± 22.8	140.0 ± 24.0	126.4 ± 28.0	164.4 ± 24.8	180.0 ± 28.0	2.09	1.00
ALFE100	1.01 ± 0.50^{b3}	1.45 ± 0.53^{b3}	1.73 ± 0.43^{b3}	1.91 ± 0.41^{b3}	2.36 ± 0.48^{b3}	40.4 ± 20.0	58.0 ± 21.2	76.4 ± 17.2	68.21 ± 16.4	94.4 ± 19.2	1.10	0.53
ALFE200	1.28 ± 0.23^{b3}	1.69 ± 0.16$^{a3,\ b3}$	1.98 ± 0.21^{b3}	2.15 ± 0.21^{b3}	3.32 ± 0.39$^{a2,\ b3}$	51.2 ± 9.2	67.6 ± 6.4	79.2 ± 8.4	86.0 ± 8.4	132.8 ± 15.6	1.54	0.74
ALFE300	1.39 ± 0.20^{b3}	2.01 ± 0.25$^{a2,\ b3}$	2.30 ± 0.49$^{a2,\ b3}$	2.76 ± 0.21$^{a3,\ b3}$	4.53 ±0.33^{a2}	55.6 ± 8.0	80.4 ± 10.0	92.0 ± 19.6	92.0 ± 8.4	181.2 ± 13.2	2.11	1.01

The urine volumes expressed as mL/100 g were calculated based on body weight of rats and urinary volumetric excretion values are expressed as a percentage of the initial hydric overload (25 mL/kg). Values are expressed as the mean ± SEM; n = 6. $^1P < 0.05$, $^2P < 0.01$, $^3P < 0.001$ versus control. ^{a}Against negative control (DW); ^{b}Against standard (Fr13). Diuretic index = volume of test group/volume of control group. Diuretic activity = urine volume of test group/urine volume of furosemide group. **DW**: Distilled water; **F13**: Furosemide 13 mg/kg; **ALFE100**: *Ammodaucus leucotrichus* fruits extract 100 mg/kg; **ALFE200**: *Ammodaucus leucotrichus* fruits extract 200 mg/kg; **ALFE300**: *Ammodaucus leucotrichus* fruits extract 300 mg/kg.

Diuretics are among the most used medications to treat certain pathologies, either of cardiac origin such as heart failure, arterial hypertension, or originating from other organs such as kidney diseases, liver cirrhosis, pulmonary edema, and toxemia during pregnancy.[22,23] They allow the elimination of water and electrolytes. Like any medication, diuretics can promote adverse effects, such as electrolyte imbalances, headaches, dizziness, changes in metabolism, alteration of sexual function, activation of the renin-angiotensin and neuroendocrine systems, and dehydration.[24]

There are different types of diuretics, each having its own mechanism of action. Scientific study continues to identify more effective molecules with fewer adverse effects. Moreover, previous animal experiments have shown that certain vegetable species widely used in traditional medicine have proven to possess diuretic effect. [6, 25] Previous studies have revealed that it is beneficial to prepare animals with various fluids before tests. Since diuretics are prescribed to treat edema, it is important to prove their effectiveness with appropriate levels of electrolytes and water.[26] To evaluate the diuretic activity of the extract, that is, its ability to treat edema, we induced this state by administering to all experimental rats a hydroelectrolytic load by oral gavage, using a saline solution (0.9% NaCl, 25 mL/kg), a procedure that has already been proven to promote edema.[27] The presence or absence of edema was not objectively demonstrated in the animals tested. The primary purpose of administering a saline load was to induce sodium and water overload in order to stimulate diuresis.

In the present study, the diuretic effect of the hydroethanol extract of *AL* fruits was examined by oral administration in normal rats, following the single administration of several doses, as this is the most widespread method of using this plant in traditional medicine among the North African population to induce diuresis. The objective was to evaluate the acute pharmacological activity of ALFE and compare it to that of the standard drug (furosemide), a reference diuretic frequently adopted in clinical practice. One of the major mechanisms of diuretic action is the increase in renal excretion of sodium as well as urine output. These effects result mainly from the inhibition of tubular reabsorption of water and electrolytes at the nephron level, thus preventing their reabsorption to the bloodstream. Furosemide, used as a reference diuretic, exerts its action by inhibiting the $Na^+/K^+/2Cl^-$ ion cotransporter located in the thick ascending limb of the loop of Henle, resulting in a significant elevation in urinary flow and sodium excretion.[28]

Effect of hydroethanol extract of Ammodaucus leucotrichus fruits on first urinary latency

The effect of the hydroethanol extract of *Ammodaucus leucotrichus* fruits on first urinary latency is illustrated in Figure 2. First, the mean values of the excreted urinary volume (UVE) obtained for the different doses of hydroethanol extract of *Ammodaucus leucotrichus* fruits was compared with those of the control. Second, these values were compared to those observed with frusemide.[29] The results showed that rats treated with different doses of hydroethanol extract of *Ammodaucus leucotrichus* fruits (ALFE 100, 200, and 300 mg/kg) passed urine rapidly at 47.83 ± 2.32, 40.67 ± 2.58, and 36.50 ± 2.17 min, respectively, which was significantly different ($p < 0.001$) compared to the negative control with first urinary latency of 57.16 ± 1.94 min. ALFE doses at all doses tested resulted in a slight slow onset of urinary excretion compared to Frusemide with first urinary latency at 25.17 ± 1.01 min (Figure 2). Urinary latency during the first two hours of the experiment played a key role as a biological marker for quantifying the pharmacological impact of the test extract.

Effect of ALFE on urine volume and urinary excretion

The effect of ALFE on urinary latency was corroborated by the results of cumulative urinary excretion (Table 1). Throughout the experiment, the animals were monitored, and particularly after the administration of the different doses of ALFE, and the rats showed no signs of dehydration. The details of the urine volume excreted by all groups of rats, after oral administration of ALFE (100, 200, and 300 mg/kg bw), the reference diuretic (furosemide), and distilled water are presented in Table 1. Oral administration of hydroethanol extract of *Ammodaucus leucotrichus* fruits increased urinary flow in a dose-dependent manner. All doses of ALFE 100, 200, and 300 mg/kg produced a significant ($p < 0.001$) increase in the volume of excreted urine, compared to the control, from the first hour, (40.4% for 100 mg/kg, 51.2% for 200 mg/kg, and 55.6% for 300 mg/kg), at 2 hours (58.4% for 100 mg/kg and 67.6% for 200 mg/kg, 80.4% for 300 mg/kg), at 4 hours (69.6% for 100 mg/kg, 79.6% for 200 mg/kg, and 95.6% for 300 mg/kg), at 6 hours (76.4% for 100 mg/kg, 79.2% for 200 mg/kg, and 92% for 300 mg/kg), and at 24 hours (94.2% for 100 mg/kg, 132.8% for 200 mg/kg, and 181.2% for 300 mg/kg). The data revealed that the reference diuretic (furosemide) significantly ($p < 0.0001$) increased urine production compared to the control at all times (123.2% at 1h; 140% at 2 h; 126.4% at 4 h; 164.4% at 6 h; and 180% at 24 h). Comparing the various doses of ALFE with each other, it was observed that the 300 mg/kg bw dose of hydroethanol extract showed significantly better diuretic activity at 1 h, 2 h, 4 h, 6 h, and 24 h than the doses of 200 and 100 mg/kg bw of ALFE. However, all three doses of ALFE promoted significant polyuria in all animals by cumulative urinary excretion at the end of the 2nd hour compared to the control. The slowing of micturition in the first urinary latency and the significant ($P < 0.001$) elevation of cumulative urinary excretion after 2 h with ALFE shows that its diuretic activity is probably caused by its secondary metabolites, it is noteworthy that the diuretic activity of the plant extract was dose- and time-dependent, indicating that the effect is intrinsic, genuine, and possibly receptor-mediated. The different doses of ALFE (100, 200, and 300 mg/kg) succeeded in producing better diuresis by significantly elevating the mean value of excreted urinary volume (UVE) compared to the control group, from the first hour until the end of the experiment. These results differ from some previous studies, particularly regarding the effect of the extract at a low dose, which showed no effect on urine volume during the first hour following its administration, contrary to what was observed in the present study. [3, 18, 30] A slight increase in diuresis was observed with doses of 100 and 200 mg/kg (2.36 ± 0.48 and 3.32 ± 0.39 mL/100mg, respectively compared to the control (2.15 ± 0.84 mL). In contrast, the highest dose (300 mg/kg) induced marked diuresis (4.53 ± 0.33 mL/100 kg), comparable to that observed with furosemide at 13 mg/kg (4.50 ± 0.70 mL/100 mg) ($p < 0.001$). However, the lower doses of ALFE produced a diuretic effect significantly inferior to that of furosemide (Table 1). The diuretic activity and diuretic action were calculated in order to compare the diuretic effect with the different groups of rats. The highest dose of ALFE (300 mg/kg) presented the highest diuretic action with a diuretic index of 2.11, which was comparable to that of Frusemide at 13 mg/kg (diuretic index = 2.09) (Table 1). In terms of diuretic activity, the extract exhibited a weak – moderate, and a dose-dependent diuretic activity. Diuretic activity is considered strong if it is above 1.50, moderate if it ranges between 1.00 and 1.50, weak if between 0.72 and 1.00, and negligible if it is below 0.72.[31] Based on the results, it can be deduced that *A. Leucotrichus* fruit extract at its highest dose, exhibits moderate diuretic activity (1.01) similar to that of Frusemide (1.0). In contrast, the activity was low at lower doses, with values of 0.53 and 0.74 for ALFE 100 mg/kg and ALFE 200 mg/kg, respectively (Table 1). The comparison of the results obtained in this study with those of previous works concerning diuretic activity and index shows a concordance, particularly at the highest dose of the extract.[3,29]

Furthermore, the highest dose of ALFE (300 mg/kg) resulted in a more pronounced urinary excretion comparable to that caused by the standard drug frusemide, particularly at 24 h (181.6 ± 13.6% versus 180 ± 26.8%). These results suggest that the extract possesses a marked diuretic activity, equivalent to that of synthetic diuretics such as Frusemide. This may be explained by the presence of secondary metabolites in the extract.[32] The acute treatment of rats with the three doses of ALFE revealed clear and significant diuresis, which seemed to be a function of the administered dose and time, suggesting the possibility of an intrinsic and causal action, which could be mediated by receptors.[23] The receptors most likely involved include vasopressin (V_2), aldosterone, and dopaminergic receptors, as well as possibly ion channels (such as Epithelial Sodium Channel ENaC and Na^+-K^+-$2Cl^-$ Cotransporter 2NKCC2) or enzymes like carbonic anhydrase, depending on the chemical composition of the extract.[33]

Effect of ALFE on urinary electrolytes excretion

In addition, the effect of the hydroethanol extract of *A. Leucotrichus*

fruits on urinary electrolytes excretion was also assessed. Urine samples from each rat group collected at the end of the experiment after 24 hours were analyzed to determine the electrolyte content (Na^+, K^+, and Cl^-). The results revealed a significant ($P < 0.001$) increase in the elimination of electrolytes. There was a significant elevation in sodium, potassium, and chloride excretion as a function of the extract dose. Although, the extract significantly stimulated the excretion of these electrolytes compared to the control group, this increase remained lower than that induced by the reference diuretic (Frusemide). The different doses (100, 200, and 300 mg/kg bw) of ALFE showed a significant elevation in the excretion of Na^+, K^+, and Cl^- compared to normal control rats. In addition, a dose-dependent increase in the Na^+/K^+ ratio was also observed (Table 2). It was observed that the saluretic index of urinary excretion of Na^+ and Cl^- of the hydroethanol extract at 300 mg/kg and Frusemide at 13 mg/kg was very close to each other (1.91; 2.04; 1.68 vs. 2.47; 2.16; 1.84, respectively). ALFE at 100 and 200 mg/kg bw revealed strong saluretic activity compared to normal control (Table 2), but ALFE at 300 mg/kg and the reference drug showed higher activity compared to ALFE at 100 and 200 mg/kg. The Na^+/K^+ ratios of ALFE100 (2.42) and ALFE200 (2.03) were higher than the ratio of the standard drug (1.86) as well as the ratio of ALFE300 (1.79). The carbonic anhydrase inhibitory activity of ALFE100, ALFE200, and ALFE300 was 0.66, 0.55, and 0.58, respectively, which were lower than that of Frusemide (Table 2).

Diuresis is characterized by two main aspects: (i) an increase in the excreted urinary volume (polyuria) and (ii) an abundant excretion of electrolytes. These effects can result from the inhibition of renal tubular reabsorption of water and electrolytes into the bloodstream.[32] It was essential, in evaluating the diuretic effect of ALFE, to measure on one hand the urine volume, and on the other hand the urinary concentrations of electrolytes which is an indication of saluretic activity.[34] In this study, the hydroethanol extract of *A. leucotrichus* fruits induced urinary hyperexcretion in a clearly dose-dependent manner. The progressive increase in doses was accompanied by a significant elevation in the excretion of electrolytes (Na^+, Cl^-, K^+) compared to the control group. Although this effect was significantly lower than that induced by the reference drug (Frusemide), it remained notable. Based on the results of urinary excretion and electrolytes obtained in this study with the various doses of hydroethanol extract of *Ammaudocus leucotrichus* fruits, it is logical to consider that the diuretic effect of the plant is of the saluretic type and similar to that caused by Frusemide, contrary to the aquaretic type, which is a typical property of the majority of phytodiuretic agents. There are several types of diuretic effects, such as aquaretic, natriuretic, kaliuretic, antikaliuretic, bicarbonaturic, and osmotic effects. Each type has its own physiological effects and a specific mechanism of action.[35] In aquaresis, the effect produced does not involve significant sodium excretion, it referred to the excretion of free water without substantial loss of electrolytes, particularly sodium. The mechanism of aquaretic effect is primarily mediated by the inhibition of the action of antidiuretic hormone (ADH, also known as vasopressin) in the kidneys.[36] The urinary concentration of Na^+ and Cl^- ions is analyzed as a key parameter for the evaluation of saluretic activity of plant extracts.[25] In this study, the different doses of ALFE (100, 200, and 300 mg/kg) significantly ($P < 0.001$) increased the excretion of Na^+ and Cl^- compared to the control. This effect could be due to the inhibition of the Na^+/Cl^- symporter (co-transporter system) in the distal convoluted tubule, by exerting competition for the Cl^- binding site and promoting hyperexcretion of Na^+ and Cl^-.[37] ALFE increased the excretion of K^+ compared to the control group, however, this increase remained significantly lower than that induced by furosemide. In this regard, the plant extract seems to contain active compounds whose kaliuresis mechanism is similar to that of the reference drug. Regarding the natriuretic activity (aldosterone secretion index) of the plant extract, it is a process that is inhibited by aldosterone. A decrease in aldosterone promotes natriuresis, whereas an increase reduces it, natriuretic activity is calculated by the Na^+/K^+ ratio. Values greater than 2.0 indicate a favorable natriuretic effect, while ratios greater than 10.0 indicate a potassium-sparing effect.[38, 39]

The index of the Na^+/K^+ ratio for doses of 100, 200, and 300 mg/kg of ALFE was 2.48, 2.03, and 1.79, respectively, and 1.86 for Frusemide (Table 2). These levels are close to the acceptable limit, and it can therefore be affirmed that the plant extract possesses good natriuretic activity. Natriuretic agents promote increased sodium excretion, which is accompanied by water loss through osmosis. Potassium-sparing agents facilitate this sodium elimination while minimizing urinary potassium loss,[35] whereas the mode of action of the hydroethanol extract does not correspond to that of potassium-sparing diuretics. The latter are characterized by low potency, a slow onset of action, and an elevation of the urinary Na^+/K^+ ratio.[22,40] In addition, the $Cl^-/[Na^+ + K^+]$ ratio allows the evaluation of the carbonic anhydrase inhibitory activity of the hydroethanol extract. Ratios between 1.0 and 0.8 suggest the absence of enzymatic inhibition. A decrease in the ratio is indicative of enzymatic inhibition.[22] ALFE presented values of 0.66, 0.55, and 0.61 at doses of 100, 200, and 300 mg/kg, respectively (Table 2). These results highlight the ability of the plant extract to inhibit the carbonic anhydrase enzyme at the level of renal tubules.

Table 2: Effect of aqueous ethanol extract of the fruits of *Ammodaucus leucotrichus* on 24 h urinary electrolyte excretion in rats

Group	Urinary Electrolyte Concentration (mMol/L)			Saluretic Index			Na^+/K^+	$Cl^-/(Na^+ + K^+)$
	Cl^-	K^+	Na^+	Cl^-	K^+	Na^+		
DW	74.16 ± 4.10	40.47 ± 1.99	88.24 ± 3.40				2.18	0.58
F13	182.93 ± 2.74[a3]	87.51 ± 3.27[a3]	162.66 ± 3.86[a3]	2.47	2.16	1.84	1.86	0.73
ALFE100 mg/kg)	117.39 ± 2.93[a3, b3]	52.31 ± 4.40[a3, b3]	126.34 ± 2.05[a3, b3]	1.58	1.29	1.43	2.42	0.66
ALFE200 mg/kg)	125.93 ±0.85[a3, b3]	64.15 ± 3.03[a3, b3]	130.52 ± 3.61[a3, b3]	1.7	1.59	1.48	2.03	0.55
ALFE300mg/Kg)	141.98 ±2.38[a3, b3]	82.69±1.04[a3]	148.19 ± 4.46[a3, b3]	1.91	2.04	1.68	1.79	0.61

Values are expressed as the mean ± SEM; n = 6. [1]$P < 0.05$, [2]$P < 0.01$, [3]$P < 0.001$ versus control. [a]Against negative control (DW); [b]Against standard (Fr13). **DW**: Distilled water; **F13**: Furosemide 13 mg/kg; **ALFE100**: *Ammodaucus leucotrichus* fruits extract 100 mg/kg; **ALFE200**: *Ammodaucus leucotrichus* fruits extract 200 mg/kg; **ALFE300**, *Ammodaucus leucotrichus* fruits extract 300 mg/kg.

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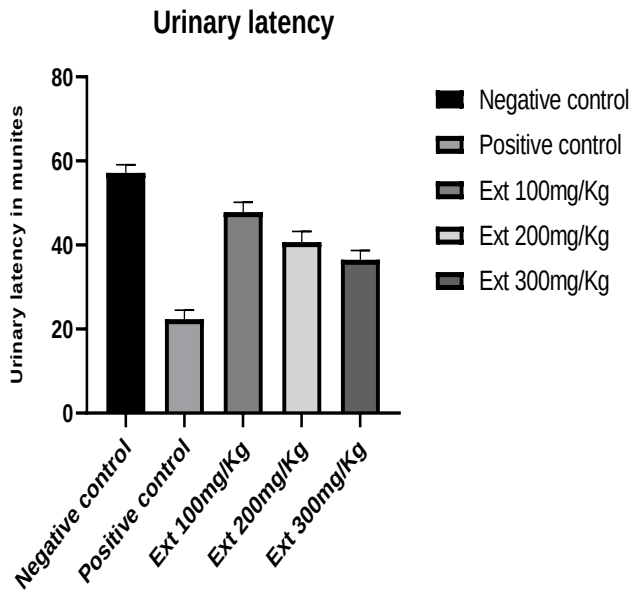

Figure 2: Effect of hydroethanol extract of *Ammodaucus leucotrichus* fruits on the first urinary latency of rats. Values represent mean ± standard error of mean (S.E.M), n = 6.

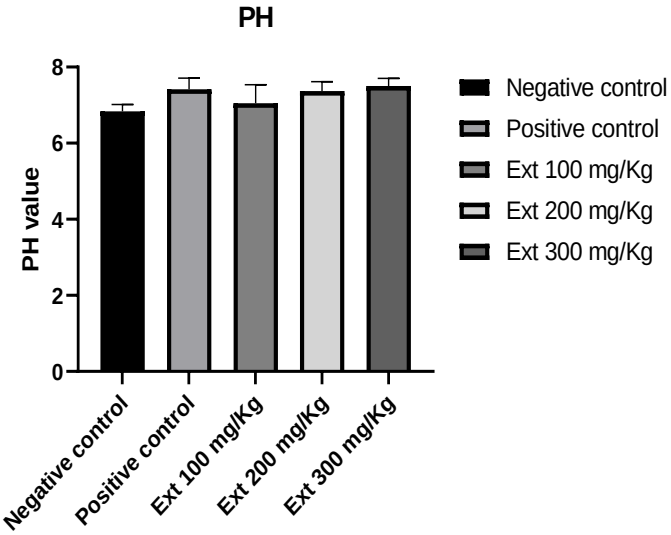

Figure 3: Effect of hydroethanol extract of *Ammaudocus leucotrichus* fruits on urine pH of rats. Values represent mean ± standard error of mean (S.E.M), n = 6.

Effect of ALFE on urine pH

The saluretic effect of *Ammaudocus leucotrichus* fruits was reinforced by a significant elevation of urinary pH in rats treated with different doses of ALFE. The pH of the groups of rats that received ALFE excreted relatively alkaline urine; 7.26 ± 0.04, 7.30 ± 0.25, and 7.50 ± 0.30 for ALFE 100; ALFE 200, and ALFE 300, respectively (Figure 3). The urinary pH of the negative control group was 6.82 ± 0.20, while the standard drug (Frusemide) increased the urinary pH to 7.2 ± 0.28, thus making the urine more basic. The elevated urinary pH suggests a reduction in the reabsorption of ions (Na^+, Cl^-, K^+) and a possible inhibition of carbonic anhydrase, an enzyme involved in maintaining acid-base balance at the renal level. In general, an alkaline urine pH typically reflects the presence of bicarbonate, evidenced by an elevated urinary HCO_3^- concentration.[41]

Overall, ALFE showed a selective diuretic activity characterized by a urinary hypoexcretion of K^+ compared to that of Na^+ and Cl^-. This specificity represents a notable pharmacological advantage over synthetic diuretics. Indeed, one of the main side effects of loop diuretics and thiazide diuretics is hypokalemia, which requires potassium supplementation either orally or by resorting to potassium-sparing diuretics, which limit the urinary loss of K^+.[38,39] In the present study, the AL extract stimulated the excretion of water as well as that of Na^+, K^+, and Cl^- ions, suggesting an osmotic mechanism of action or one similar to that of loop diuretics. It is therefore probable that the observed diuretic effect results from an inhibition of tubular reabsorption of water and electrolytes, a mechanism already reported for several extracts of plant origin.

Acute toxicity of ALFE

The acute toxicity effect of ALFE administered at a dose of 2000 mg/kg on body weight over a 15-day period, was evaluated via comparison of two groups: the extract-treated group (ALFE at the dose of 2000 mg/kg) and a baseline control group (control). Body weight was used as an indicator of general health and potential toxic effect of the plant (Figure 4). In the ALFE group, represented by red circles, there was a general trend of fluctuating body weight throughout the study period. Initially, the body weight showed a slight increase, reaching a peak around day 7. This peak could suggest an initial physiological response to the extract. After this point, there was a gradual decline in body weight, but it stabilized and remained relatively consistent until the end of the observation period. The error bars around the data points suggest variability in the response among the subjects, but overall, the weights did not drop below the baseline level, indicating that the extract at 2000 mg/kg did not cause a significant reduction in body weight that might suggest severe toxicity.

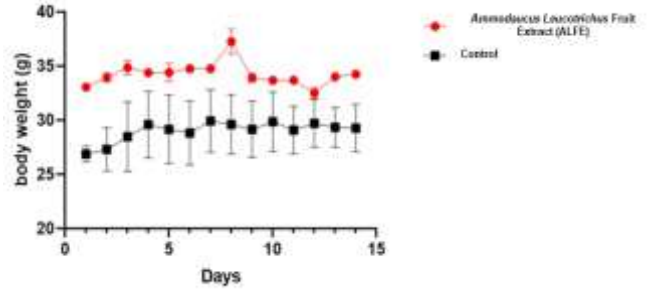

Figure 4: Body weight of mice treated with *Ammodaucus leucotrichus* fruit extract at 2000 mg/kg during the acute toxicity test

The lower and more consistent body weights in the control group could be attributed to the absence of any intervention, serving as a control to compare the potential effects of the plant extract. Overall, the data suggests that the plant extract at a dose of 2000 mg/kg did not cause any apparent sign of toxicity as evidenced by the absence of a drastic decrease in body weight, and other clinical manifestations such as tremors, lethargy, paralysis, signs of stress, or behavioral alterations. Furthermore, no cases of diarrhea were observed, and no deaths were recorded within the treated groups, suggesting that the LD_{50} is greater than 2000 mg/kg. However, the observed fluctuations in the body weight of the ALFE group imply that while the extract may not cause severe toxic effects, it may still have some impact on the physiological state of the animals, warranting further investigation to fully understand its safety profile. On the other hand, a previous study conducted to evaluate the acute toxicity of the essential oil of *Ammaudocus leucotrichus* indicated that the maximum tolerated dose (MTD) of the

essential oil of this plant was 200 mg/kg, while the LD_{50} was estimated between 520 and 570 mg/kg. These data suggest a low toxicity of this essential oil, an important parameter to take into account in the choice of doses used in humans.

Chemical profile of ALFE

Gas chromatography–mass spectrometry (GC-MS) analysis of the *Ammodaucus leucotrichus* extract obtained through cold maceration revealed a chemically diverse profile. The GC-MS analysis identified 13 chemical compounds with molecular weights ranging from 130 to 390 g/mol and retention times between 5.124 and 45.404 minutes. The resulting chromatogram is presented in Figure 5. Detailed information on the identified compounds including molecular weight (MW), chemical name, retention time (RT), peak area, and molecular formula is presented in Table 3.

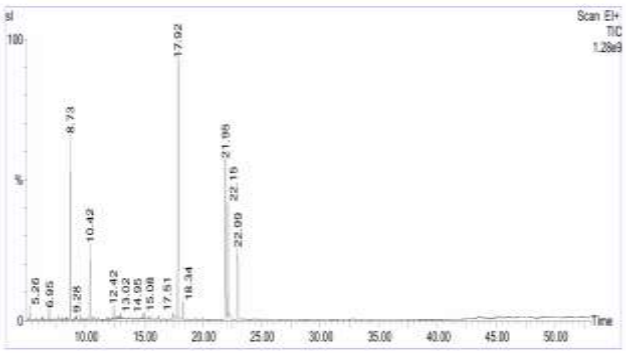

Figure 5: GC Chromatogram of the hydroethanol extract of *Ammodaucus leucotrichus* fruits

Table 3: Compounds identified by GC-MS analysis of *Ammaudocus leucotrichus* **fruit** extract

Peak	Compound Name	RT (min)	Peak Area (%)	Molecular weight	Molecular formula
1	Pentanoic acid, 4-oxo-, methyl ester	5.258	2.56	130	$C_6H_{10}O_3$
5	Benzaldehyde, 3,4-dimethyl-	8.734	38.04	134	$C_9H_{10}O$
18	Methyl tetradecanoate	15.084	1.49	242	$C_{15}H_{30}O_2$
21	Pentadecanoic acid, methyl ester	16.322	1.08	256	$C_{16}H_{32}O_2$
22	Naproxen methyl ester	17.51	2.51	244	$C_{15}H_{16}O_3$
25	Hexadecanoic acid, methyl ester (Palmitic acid)	17.918	100.0	270	$C_{17}H_{34}O_2$
26	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	18.339	6.56	292	$C_{18}H_{28}O_3$
29	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (Linoleic acid)	21.954	84.08	294	$C_{19}H_{34}O_2$
30	9-Octadecenoic acid (Z)-, methyl ester (Oleic acid)	22.154	77.15	296	$C_{19}H_{36}O_2$
32	Methyl stearate (Stearic acid)	22.988	42.79	298	$C_{19}H_{38}O_2$
35	Eicosanoic acid, methyl ester	32.797	1.59	326	$C_{21}H_{42}O_2$
42	Betulin	44.353	1.47	442	$C_{30}H_{50}O_2$
46	Diisooctyl phthalate	45.404	4.42	390	$C_{24}H_{38}O_4$

Conclusion

The results of the present study confirm the traditional use of *Ammaudocus leucotrichus* as a diuretic agent. The hydroethanol extract of the fruits (ALFE) demonstrated a significant capacity to induce diuresis, promoting the elimination of water and electrolytes. These results corroborate both popular uses and ethnobotanical data reporting the diuretic properties of this plant. Nevertheless, more in-depth scientific investigations are necessary to isolate and characterize the bioactive compounds responsible for this effect. To date, no previous study has been conducted in this field.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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