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Phenolic Profile, Anti-Inflammatory and Diuretic Properties of Asplenium Ceterach Tested on Albino Mice and Wistar Albino Rats

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Abstract- Asplenium ceterach is a fern used in traditional medicine against kidney stones and as a diuretic agent. Thus, we set as objective of this study is the determination of the phenolic profile as well as the study of anti-inflammatory and diuretic properties of *Asplenium ceterach* (Rustyback). Phenolic composition is achieved by High-performance liquid chromatography, the anti-inflammatory activity is determined in albino mice, and the diuretic activity in Wistar albino rats. The results of phenolic profile show an overall concentration of 3926 µg/g DM of *Asplenium ceterach*, of which 3/4 are represented by chlorogenic acid. This plant is endowed with an interesting plantar anti-inflammatory property and a topical anti-inflammatory comparable to that of ibuprofen. *Asplenium ceterach* expresses an excellent diuretic activity significantly higher that of Furosemide. This study confirms the traditional use of the infusion of *Asplenium ceterach* as a powerful diuretic and an anti-inflammatory.

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I. Introduction

Several researches have intensified on medicinal plants used in therapeutic purposes subsequent the evolution of means and methods of analysis. The special interest for these plants is their ability to synthesize and accumulate micronutrients endowed with preventive and curative properties of innumerable pathologies.

Many substances of plant origin, such as phenolic compounds, terpenoids, and alkaloids are sought for their variables biological activities and therapeutic properties for several diseases including cardiovascular disorders, cancers, and neuro-degenerative diseases (1).

The plant which is the subject of this study is *Asplenium ceterach* (Finger fern and locally called "Tahchicht waman tassa" or "Kessar lahjar") and which is belongs to Aspleniaceae family known as a common plant of rocks and old limestone walls. This perennial plant is characterized by 5-15 cm of length, short strain, thick, and turf. Its leaves are tufted, rolled in lacrosse at their young age, spread, with a short and scaly petiole, narrow oblong, pennatized, short-lobed, ovate-obtuse, whole or crenate, alternate, confluent, thick, glabrous, and green on top, covered under shiny scales first silvery then reddish. The fructification is arranged on the underside of the leaves in elongated, straight, scattered and placed obliquely on the midrib, devoid of indusia. It adapted to drought; in dry weather the leaves curl and the presence of scales disposed in the under face limited evapotranspiration (2).

The medicinal plant *Asplenium ceterach* is little studied, however, some properties and traditional uses have been reported as its use against kidney stones, diarrhea, neuralgia as well as prostate and gastrointestinal disorders (3). This plant is also endowed with interesting antioxidant potential and antimicrobial and protective activities against DNA damage (4). The infusion prepared with this plant is an excellent diuretic by taking 2 to 3 cups per day (5).

In speaking of diuretic, diuresis is a vital function for the living being by which the organism excretes all the waste resulting from the cellular combustion, once it has kept the necessary substances to feed itself and produce energy, it is considered among the functions of elimination. Many diseases, namely coronary heart disease, arterial disorders, cardiac, and renal insufficiency, are related to hypertension (6,7). Common clinical strategies to reduce blood pressure include the prescription of drug that decreasing arterial resistance and/or reducing cardiac output. Among the medicament most used to promote increased urine volume output, urinary sodium, and which leads to the reduction of blood pressure are diuretics (8).

Inflammation is the response of vascularized tissue to physical, chemical, or biological aggression in order to maintain its integrity. Inflammatory response is a usually beneficial process; its goal is to mobilize the immune system to eliminate the pathogen and repair damaged tissues. In some cases, inflammation may be harmful because of the aggressiveness of the pathogen, its persistence, the site of inflammation, or abnormal regulation of the inflammatory process (9). The use of drugs and some plants reduce inflammation and thus prevent pain.

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To our knowledge, no work has been conducted to investigate biological activities of *Asplenium ceterach*. Thus the objectives of this investigation are the determination of phenolic profile as well as anti-inflammatory and diuretic properties of *Asplenium ceterach*.

II. Materials and Methods

a) Plant material

Asplenium ceterach was collected from Fenaia Ilmaten municipality (Bejaia department, Algeria) at an altitude of 400-450m during the period from March to April 2017. The aerial part of the harvested plant was dried in the open air during 15 days after which the dried plant was crushed and reduced to a fine powder using an electric grinding mill (Philips, Enapem®) and then sieved using a sieve of mesh size of 250 μm. The resulting powder is kept away from air and moisture.

b) Preparation of plant extracts

Extraction of phenol compounds is carried out by maceration of the *Asplenium ceterach* powder (1 g) using 10 ml of methanol HPLC grade with magnetic stirring during 30min. The extract is separated from the powder by filtration. The powder is re-extracted two more times using 10 ml with the same solvent. The three recovered filtrates are mixed and the solvent is removed by a rotary evaporator using the temperature of 65 °C until the volume is reduced to 4 ml.

c) Phytochemical screening

Phytochemical screening is a set of tests carried out either on the powder or on the plant infusion to highlight the presence or absence of certain secondary metabolites. Phytochemical screening of *Asplenium ceterach* concerns flavonoids, anthocyanins, leucoanthocyanins, total tannins, catechic tannins, gallic tannins, saponosides, coumarins, free quinones, combined quinines, and alkaloids (10,11).

d) Determination of phenolic compounds

The phenolic profile of *Asplenium ceterach* is determined by high performance liquid chromatography (Waters 2695 Alliance) equipped by a diode array detector (DAD) set at three wavelengths (280, 320, and 350 nm). For the elution of phenolics, a mobile phase consisting of 1% formic acid solution (A) and acetonitrile (B) that used with a flow rate of 1 ml/min. For the stationary phase, it consists on LiChrosorb RP-18 column (250 x 4.0 mm, 5 µm). The volume of extract injected is 10 µl and the system is set at a temperature of 30 °C. The elution gradient used is as follows: 5% (B): during the first two minutes, 02-12 min: 5-95% (B), 12-12.2 min: 95-5% (B), and 5% (B) up to 20 min. Phenolic compounds are identified and quantified by reference to retention times and peak areas of phenolic standards and the results were expressed as micrograms per gram of dry matter (µg/g DM).

The total phenolic content (TPC) is determined by the Folin-Ciocalteu method according to Singleton and Rossi (12). The TPC is expressed as µg/g DM according to the calibration curve using gallic acid as standard ($y = 11.64x - 0.019$, $R^2 = 0.998$).

e) Biological properties

i. Animal material

15 Albino female/male mice (weighing between 26-28 g) and 15 Wistar albino male rats (weighing from 150 to 200 g) obtained from the pharmaceutical group company SAIDAL (Algiers, Algeria) are used for anti-inflammatory and diuretic activities. Animals were housed in plastic cages with a 10/14h light/dark cycle at an ambient temperature of 20-24°C. Animals were fed standard diet and water *ad libitum*. All experiments were in compliance with the guidelines for the care and use of laboratory animals published by the US National Institute of Health (NIH publication No 85-23, revised 1985) with approval of SAIDAL ethic committee.

ii. Anti-inflammatory activity

Anti-inflammatory activity is estimated according to Vetriselvan et al. (13). The fifteen mice are randomly divided into three groups (n = 5). The first group received orally 0.5mL of *Asplenium ceterach* extract (20g of plant powder extracted by 100ml of boiling distilled water per 15min); the second is treated with 0.5mL of Ibuprofene (a diuretic drug prepared at 200mg/250mL dw) and considered as a positive control; the third group (negative control) received 0.5mL of distilled water.

Subsequently, the plantar and topical inflammations are induced after 30 min. The plantar inflammation is generated by the injection of carrageenan (25µl) under the plantar aponeurosis of the left hind paw of the mouse; the no treated right paw is considered as a control. The topical inflammation is induced by topical application of xylene (10µl) on outer surface of right ear of each mouse; the left ear represents the control.

The mice are sacrificed by cervical dislocation after 4 h of the inflammatory induction. The paws and ears are removed from the same position and weighed in analytical balance immediately. The swelling degree (SD) was estimated as the difference in the weight between treated and untreated limbs and the inhibitory rate was calculated as follows:

$$\text{Inhibitory rate (\%)} = 100*(SD_{Control} - SD_{Treated})/SD_{Control}$$

iii. Diuretic activity

Diuretic activity is determined according to the method described by de Paula Vasconcelos et al. (14). The 15 Wistar albino male rats were randomly divided into three groups (n = 5). Before treatment all animals were fasting 18 hours. Then, *Asplenium ceterach* extract was administered by the oral route at dose of 50 mg/kg. The negative control group received the isotonic saline

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(0.9% NaCl, 50 ml.kg) and the positive control group is treated with 50 mg/kg of Furosemide. Urine was collected and measured for each rat and averaged for every group after each hour during 6h and the cumulative urinary excretion (CUE) was calculated.

III. Results and Discussion

a) Phytochemical screening

The results of the phytochemical screening carried out on the infused and the powder of *Asplenium ceterach* are summarized in *Table 1*. The tests indicate that the studied plant contains many classes of secondary metabolites, of which leucoanthocyanins and tannins are the most abundant. Free quinones, coumarins, and flavonoids are also present with appreciable levels. However, the phytochemical screening does not reveal the presence of anthocyanins, combined quinones, saponosides, and alkaloids.

Table 1: Phytochemical screening results of *Asplenium ceterach*.

Metabolites	Results
Flavonoids	+
Anthocyanins	-
Leucoanthocyanins	+++
Total tannins	+++
Catechic tannins	+
Gallic tannins	++
Saponosides	-
Coumarins	+
Free quinones	+
Combined quinones	-
Alkaloids	-

(-), Absence of metabolites; (+), low level; (++), medium; (+++), high level.

b) Phenolic profile of Asplenium ceterach

Seven phenolic compounds are identified in studied plant and results are regrouped in Table 2. Chlorogenic acid is the compounds with the higher concentration (2817.72µg/g DM) which represents ¾ of total phenolics of *Asplenium ceterach*. This latter contains appreciable content of gallic, vanillic, and coumaric acids as well as low concentrations of quercetin and benzoic acid. Overall, total phenol content of *Asplenium ceterach* is 3926.19µg/g DM, indicating the richness of this plant on phenolic compounds.

Table 2: Identification and quantification of phenolic compounds of *Asplenium ceterach* by HPLC-DAD

Compounds	Retention time (min)	Wavelength (nm)	Peak area	Concentration ($\mu g.g^{-1}$ DM)
Hydroxybenzoic acids				
Gallic acid	3.484	280	8657895	829.56
Vanillic acid	8.519	350	112851	123.15
Benzoic acid	13.088	280	51459	6.88
Tannic acid	15.091	280	1055650	32.14
Hydroxycinnamic acids				
Chlorogenic acid	6.899	320	29407706	2817.72
Coumaric acid	7.950	320	3204610	100.80
Flavonoids				
Quercetin	10.950	280	88687	15.94
	Total			3926.19

TPC of *Asplenium ceterach* is also quantified using Folin-Ciocalteu method. This latter is widely used for the overall quantification of phenolic compounds. It based on the oxido-reduction reaction between the hydroxyl groups of the phenolics and the phosphotungstic and molybdic acids of the Folin-Ciocalteu reagent which it reduction causes the change of its color from greenish-yellow to blue. The result of total phenolic content obtained using Folin-Ciocalteu essay of the studied plant revealed a value of 7466.67 ± 148.49 µg/g DM.

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The comparison of TPC results using HPLC and Folin-Ciocalteu reagent indicates that the concentration obtained by the second method is higher than the first one. This could be explained by the fact that Folin-Ciocalteu essay measures through the oxido-reduction reaction all compounds present in the extract that allowing the reduction of this reagent (phenolic compounds, reducing sugars, amino acids, etc.), whereas the chromatographic method is more accurate because it targets the compound of interest.

c) Anti-inflammatory property

The evaluation of the anti-inflammatory activity of *Asplenium ceterach* is conducted by induction of acute inflammation on the paws and ears of the mice using carageenin and xylene, respectively. Inflammation is measured by changes in paw and ear weights following induction or inhibition of edema formation. Four and a half hours after treatment with carageenin and xylene solutions, the mice developed edema at their left paws and right ears. The difference in weight compared to the weights of uninflected right legs and left ears represents the formed edema.

From control results, it indicated that the use of inflammatory agents induced significant increase of legs and ears weights by the accumulation of edema *(Table 3)*. The administration of *Asplenium ceterach* infusion to mice produced an interesting decrease of the inflammatory symptom. The drug used as a reference (Ibuprofen 200 mg) diminished topical and plantar inflammations by a half. *Asplenium cetarach* extract presents a good plantar anti-inflammatory activity (32.75%) and an topical inflammatory property close to that of Ibuprofen 200mg.

Table 3: Anti-inflammatory activities results of control, *Asplenium cetarach*, and Ibuprofen 200 mg.

Group	Parameter	Right legs (g)	Left legs (g)	Left ears (g)	Right ears (g)
Control	Average weight	0.076	0.109	0.029	0.048
	Standard deviation	0.011	0.011	0.004	0.005
	% of edema	*44.33%*		*64.14%*	
Asplenium ceterach	Average weight	0.127	0.165	0.032	0.044
	Standard deviation	0.009	0.007	0.004	0.010
	% of edema	*29.81%*		*35.80%*	
	% of edema reduction	32.75%		44.18%	
Ibuprofen 200 mg	Average weight	0.139	0.169	0.159	0.210
	Standard deviation	0.021	0.018	0.027	0.031
	% of edema	*21.58%*		*31.78%*	
	% of edema reduction	51.31%		50.44%	

The anti-inflammatory activity of *Asplenium ceterach* could be strongly due to the richness of this plant on chlorogenic acid which represents 3/4 of the total content of phenolic compounds. Several studies have shown that chlorogenic acid has anti-inflammatory properties (15,16). Hwang *et al.* (17) found that chlorogenic acid significantly inhibited not only NO production but also the expression of cyclooxygenase-2 and NO synthase. This compound also attenuated pro - inflammatory cytokines such as IL-1β and TNF-α and other inflammation-related markers. Chlorogenic acid decreased also the endotoxin-induced adhesion of macrophages, the expression level of ninjurin1 (Ninj1), and the nuclear translocation of NF-κβ.

d) Diuretic activity

The diuretic activity of *Asplenium ceterach*, tested on Wistar albino rats, is compared to those of the control (0.9% saline solution) and the reference (diuretic drug: Furosemide 40mg). The accumulated volumetric urinary excretion of *Asplenium ceterach* increases continuously during the 6 hours with a great intensity during the first 3 hours and then the excretion rate decreases slightly *(Figure 1)*. For the control, the urinary excretion increases from the 1st to the 2nd hour then resumes continuously from the 3rd hour. Concerning the group treated with Furosemide, the excretion begins slightly; afterwards it becomes very intense between the 2nd and the 4th hours, and stops during the last two hours. The total urinary volumes indicate that the treatment with *Asplenium ceterach* infusion gave the highest diuretic activity, with a volume of 7.1ml, compared to the control (5.2ml) and the reference (5.7ml) which correspond to volumetric urinary excretions of 83.53, 69.32, and 76.00%, respectively.

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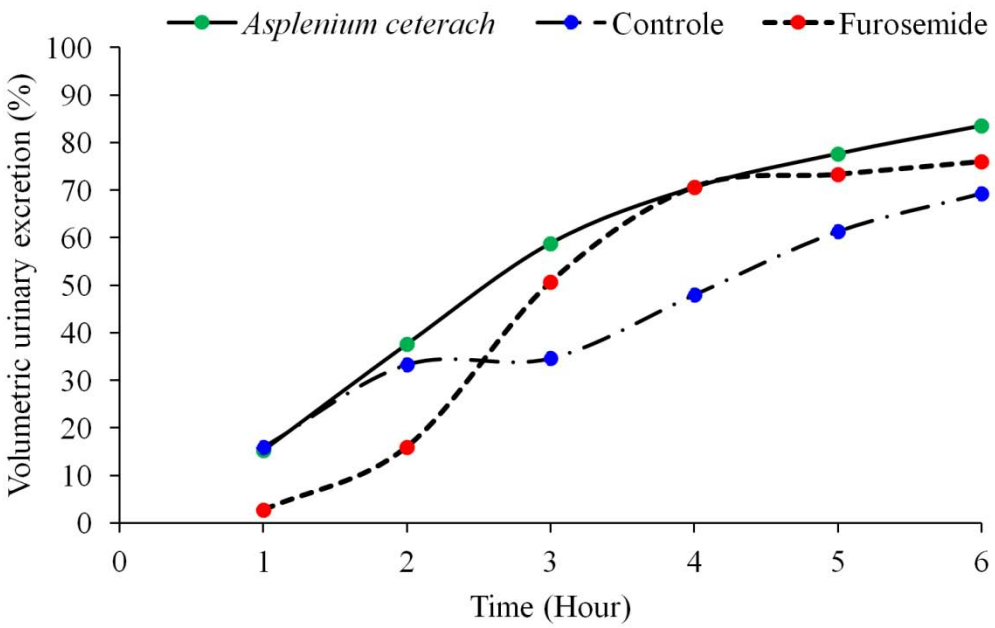


Figure 1: Accumulated volumetric urinary excretion (%) as a function of time.

The diuretic activity has been demonstrated by several other plants such as *Merremia emarginata* and *Hibiscus sabdariffa* which is due to the presence of chlorogenic acid (18,19). During the catabolism of this latter, hippuric acid was formed that can act as diuretic agent but the mechanism of this compounds still unclear (18).

IV. Conclusions

This study concludes that *Asplenium ceterach* is characterized by a particular phenolic profile with the dominance of chlorogenic acid. Animal tests (mice and rats) have shown that this plant is endowed with interesting anti-inflammatory and diuretic properties.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding this work.

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