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Phytochemical Screening and evaluation of Heavy metals in a medicinal plant *Marrubium vulgare L*

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Abstract— Our research is part of the enhancement of our plant heritage that we deemed necessary to exploit it, our choice fell on a medicinal plant *Marrubium vulgare L* and this by the phytochemical characterization (of leaves, stems and roots) followed by the study of the xerophytic character, by determining the content of five heavy metals (Zinc, Copper, Cadmium, Lead and Chromium)in the different organs and by analysis with atomic absorption spectrometry (AAS). The results of the phytochemical tests showed the richness in secondary compounds, namely: flavonoids, tannins, saponosides, coumarins and reducing compounds. heavy metal analyzes showed that *Marrubium vulgare L* was found to have a high capacity to accumulate heavy metals, especially chromium and copper. The levels of its heavy metals are within the toxic threshold of plants.

Keywords—chromium, extraction, *Marrubium vulgare L*, , phytochemical.

I. INTRODUCTION

Algeria is rich in aromatic and medicinal plants that can be used in different fields (pharmacy, perfumery, cosmetics, food) for their therapeutic, organoleptic and fragrant properties. These aromatic plants are at the origin of products with high added value (essential oils, extracts, resins, etc.) which almost always appear as complex mixtures [1]. Medicinal and aromatic plants have been recognized as an important resource for health care and perfumery since antiquity [2].

Secondary metabolites that find uses as flavoring agents, perfumes, insecticides, dyes and drugs. Biotechnology offers several choices by which the secondary metabolism of medicinal plants can be modified in innovative ways, to overproduce phytochemicals of interest, to reduce the content of toxic compounds or even to produce new chemicals [3].

Medicinal plants are known for their antifungal, antibacterial, antioxidant, antiviral and medicinal properties [4]-[6].

Marrubium vulgare L is one of the oldest Algerian medicinal plants belonging to the genus Marrubium known for its therapeutic properties: antifungal, antibacterial, insecticide and other biological activities [7]. Known since the highest antiquity, it is used by traditional medicine for the treatment of different types of human pathologies, in particular those related to respiratory, inflammatory, painful processes, and those of diabetes mellitus. In North Africa and Algeria, it is used as an infusion for antihypertensive therapy, as an expectorant, in antispasmodic therapy for acute or chronic bronchitis, coughs and colds and in asthma, loss of appetite and dyspepsia [8], [9].

Heavy metals cannot be biodegraded and therefore persist in the environment for long periods and is a daily hazard [10]. Among the methods of treating polluted soils used, we distinguish chemical treatment, biological treatment, and joint remediation [11], [12]. Medicinal plants can accumulate heavy metals from the soil, posing a significant problem relating to contamination in the food chain [13].

For this, in this study we are interested in the valorization and the exploitation of medicinal plants of the region of Saida, it is about *Marrubium vulgare L*.

In this work, we have set the following objectives:

-Phytochemical screening aims to characterize the presence of families of chemical compounds in the different parts of *Marrubium vulgare L* (leaves, stems and roots).

- Evaluation of the xerophytism character of *Marrubium vulgare L* by determining the contents of heavy metals by atomic absorption spectrometry.

The method of work, which we have adopted, is based on a multidisciplinary action, aiming at the valorization of the material vegetable. This method makes it possible to link the elements of information provided by the botanists at work of chemists and biologists.

II. MATERIALS AND METHODS

Plant material

The plant material of our study consists of the aerial parts: leaves and stems on the one hand and on the other hand the roots of *Marrubium vulgare L.* (fig. 1).

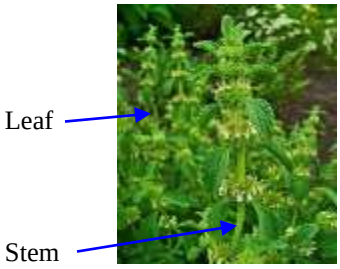

Fig. 1 Photo of *Marrubium vulgare L*

Harvest period

Marrubium vulgare L was harvested locally in the region named Ain El Hadjar located in the city of saida-Algeria, in the period February - March 2019 in Saida.

The botanical identification of this species was made in Tlemcen at the Laboratory of Ecology and Management of Natural Ecosystems (LEGEN) and confirmed by Professor Bennabadji (Abou Bekr Belkaid University of Tlemcen - UABBT).

II.1 Preparation and preservation of plant material

The harvested plants were cleaned, washed with tap water then with distilled water, dried at room temperature in the shade, in order to preserve the maximum integrity of the molecules targeted by our study by minimizing the various fermentation and degradation mechanisms inherent in the organic nature of this raw material. Once the drying operation was completed, the parts intended in particular for the leaves, stems and roots were separated, then weighed, roughly crushed and kept in glass bottles in order to carry out the various tests.

II.2 Determination of water content (moisture)

Dry the empty capsules in an oven for 15 minutes at 103° ± 2°C then leave to cool in a desiccator. Weigh 2g of sample in each previously tared capsule and place them in the oven set at 103 ± 2°C for 3 hours then remove the capsules from the oven and place them in the desiccator. Weigh the capsules after cooling. The operation is repeated until a constant weight is obtained (reduction of the drying time to 30 min).

-Expression of results (1):

$$H(\%) = \frac{M1 - M2}{P} \quad (1)$$

With : H%: water content or humidity ; M1: the initial mass in g: fresh material + capsule before drying ; M2: the final mass in g: dry matter + capsule after drying. P: the mass of the test sample in g. The dry matter content is calculated according to (2): Dry matter (Ms)=100-H% (2)

II.3 Phytochemical examination or test

All parts (leaves, stems and roots) of *Marrubium vulgare L* were subjected to phytochemical tests. During the latter, three extraction solvents of different polarities (water, diethyl ether and ethanol) are used. This qualitative study based on coloring and precipitation reactions by specific chemical reagents. Characterization tests of the different chemical groups have been carried out according to the references [14]-[18]. After each extraction, we calculated the yield of each residue obtained according to the following formula (3) :

$$R(\%) = \frac{M}{M0} \quad (3)$$

R: Yield expressed in %; M: Mass in grams of the dry extract resulting after evaporation of the solvent ; M0: Mass in grams of plant material to be treated.

II.4 Mineralization

First step: pre-mineralization (or calcination)

200 to 300 mg ± 0.1 mg of vegetable powder are weighed into a porcelain capsule. The capsule is placed in a muffle furnace. The best results are obtained by keeping the oven at 300°C until the carbon ceases to glow. The oven temperature then rose to 400-500°C. The mineralization time is variable, it depends on the nature of the material to obtain friable white ashes. The oxidation of the last traces of organic material is then carried out by adding 1 to 2 mL of HNO_3 (1N) after cooling the capsule. It is evaporated to dryness on a heating plate or in a sand bath and placed in the oven at 400° C. for one hour.

Second step: dissolving the heavy metals

Dissolution for the determination of heavy metals was carried out by acid attack of a quantity of 1g ± 0.1mg of plant sample per (10 ± 0.1) mL of aqua regia (7.5 ± 0.1mL of 37% (m/m) HCl, density 1.19g.mL-1, Merck Supra pure max Hg 0.005 ppm and and 2.5 ± 0.1mL of 65%(m/m) HNO_3, ρ=1.38g.m/L, Merck Supra pure max Hg 0.005 ppm, by a volume ratio (3/1) Then everything is boiled on a hot plate for 3 hours by a heating system closed (using reflux heating). After cooling, filter into a (25 ± 0.1) mL volumetric flask and then fill up with distilled water to the mark.

Expression of results

The determination of the metallic trace elements of our samples was carried out by atomic emission spectrometry (SSA). The results obtained were expressed (4) in ppm (mg of metal/kg of dry matter).

$$T = \frac{C.V}{S} \times D \quad (4)$$

Where : T: content of the element in mg/Kg (or ppm) ; C: concentration of the element in mg/L (given by the device after calibration) ; S: earth electrode weight in g ; V: volume of extraction (or dissolution) expressed in mL (V= 25mL), D: dilution factor.

III. RESULTS AND DISCUSSIONS

III.1 Moisture content or water content (%)

The result obtained shows that the powder of the stems, leaves and roots lost almost: 9, 5 and 1.4% respectively of their mass weight during desiccation. The results are all below 10%, from which it can be deduced that these results comply with the standards required by the European Pharmacopoeia [19]. H (%) Leaf > H (%) Stem > H (%) Root.

III.2 Dry matter (Ms)

Ms (roots)=98.6% > Ms (Leaves)= 95% > Ms (leaves)=91%.

III.3 Determination of extraction yield (R%)

The extraction yields were determined by the following formula (5) :

$$R\% = \frac{\text{Mass of the residue obtained}}{\text{Mass of vegetable powder used}} \times 100 \quad (5)$$

For each sample, we calculated the extraction yield, the results obtained are presented in table1.

Table 1. Operative data of extractions carried out on the leaves, stems and roots of *Marrubium vulgare L* .

Part used	Mass (g)	Solvent used	Appearance of the extract	Mass of extract (g)	Yield (%)
Leaves	20	Diethyl ether	Viscous	3.00	15
Leaves	20	Ethanol	Viscous	1.2	6.0
Leaves	20	Water	Viscous	2.7	13.5
Stems	20	Diethyl ether	Viscous	0.6	3.0
Stems	20	Ethanol	Viscous	0.3	1.5
Stems	20	Water	Viscous	1.8	9.0
Roots	20	Diethyl ether	Viscous	0.6	3.0
Roots	20	Ethanol	Viscous	1.2	6.0
Roots	20	Water	Viscous	0.6	3.0

The highest extraction yields that have been obtained are:the ethanolic extract of the leaves (15%) followed by the aqueous extract of the stems for *Marrubium vulgare L.*

III.4 Phytochemical screening

Phytochemical tests are carried out on extracts from the leaves, stems and roots of *Marrubium vulgare L* prepared in different solvents (water, ethanol and dithyl ether) by a single method of preparation (decoction).

The results of phytochemical tests of aqueous extracts, of ethanolic extracts and ether extracts are given in table 2.

+++ : Strongly positive ; ++ : Moderately positive; + : Weakly positive ; - : Negative .

Table. 2: Results of phytochemical tests

Chemical family	Unitary part	Results
Saponosides	Leaves	++
	Stems	++
	Roots	++
Tannins	Leaves	+
	Stems	+
	Roots	+
Salt alkaloid	Leaves	++
	Stems	++
	Roots	++
Flavonoids	Leaves	++
	Stems	+
	Roots	+
Catechical tannins /gallic Compounds reducers	Leaves	++
	Stems	+
	Roots	+
	Leaves	+++
	Stems	+
	Roots	+
Coumarin	Leaves	++
	Stems	++
	Roots	++
Sterols and Steroids	Leaves	++
	Stems	++
	Roots	++
Fatty acids	Leaves	-
	Stems	-
	Roots	-

The phytochemical screening allowed us to highlight the presence of some secondary metabolites namely: tannins, flavonoids, alkaloids, reducing compounds, coumarins, sterols and steroids, saponosides, starches and emodols. On the other hand, the tests of free anthraquinones and fatty acids are marked negative. Similarly, we recorded that the ethereal extract is very low in secondary metabolites.

III.5 Dosing of heavy metals in *Marrubium vulgare L.*

Total ash content (CT)

It characterizes the quantity of residual substances not volatilized when the drug sample is completely calcined (table 3) [20].

The table above shows that: CT (%) root > CT (%) leaf > CT (%) stem. It is clearly seen that the roots and the leaves contain the same amounts of total ash.

Content of metals in *Marrubium vulgare L*

The dosing of heavy metals such as: Pb, Cu, Cd, Zn and Cr was carried out by atomic absorption spectroscopy (AAS). Thus, all the results are given in $mg.L^{-1}$ and in order to compare them with the various international standards, all the values have been converted into $mg.kg^{-1}$ of dry matter (or ppm) (table 3).

Table 3 . The contents in ppm (mg/kg of Ms) of heavy metals in the different organs of *Marrubium vulgare L.*

Metal	organ	The contents (mg/kg of Ms)	International standards (mg/kg of Ms) [21]

	Leaves	74.49	Toxicity : 100-400
Zn	Stems	28.98	Deficiency : 15-20
	Roots	123.16	Normal content : 50
	Leaves	0.80	Toxicity : 12-300
Pb	Stems	2.39	Deficiency : -----
	Roots	75.68	Normal content : 1.0
	Leaves	19.37	Toxicity : 3
Cr	Stems	45.88	Deficiency : -----
	Roots	54.40	Normal content : 1.50
	Leaves	19.44	Toxicity : 20-50
Cu	Stems	21.40	Deficiency : 3-5
	Roots	38.20	Normal content : 10
	Leaves	2.80	Toxicity : 5
Cd	Stems	2.27	Deficiency : ----
	Roots	2.74	Normal content : 0.01-1

----- **:** value not given (not determined)

Variation and toxicity of heavy metals in the different organs

These results will allow us to determine on the one hand the contamination of the plants and on the other hand the rate of accumulation in their different parts.

✓ Zinc and lead

It can be seen that zinc and lead are distributed in a variable manner in the different parts of *Marrubium vulgare L* where they are mainly concentrated in the roots, the levels of which exceed the minimum toxicity threshold of the plants. So we have a fairly strong accumulation of zinc and lead in the roots. Zinc levels in organs can be classified as follows:

[Zn] roots > [Zn] leaves > [Zn] stems.

Lead is located as follows : [Pb] roots > [Pb] stems > [Pb] leaves. By comparing the results obtained between the roots and the aerial parts, it can be noted that the zinc is well transferred to the roots, continues to migrate towards the aerial parts and is therefore enriched in the stems. The zinc content in the leaves greatly exceeds the normal threshold for plants. Consequently lead are well absorbed in the roots, they remain blocked and they are little transferred in the stems and the leaves. Thus, *Marrubium vulgare L* shows no contamination by zinc and lead but a rather strong accumulation by zinc in the stems.

✓Chromium

Chromium is mainly concentrated in the roots and then the stems.The sequence of accumulation is as follows: [Cr] roots > [Cr] stems > [Cr] leaves.

All recorded chromium levels greatly exceed the critical plant toxicity level. So the *Marrubium vulgare L* is contaminated with chromium.

✓ The copper

It is mainly concentrated in the roots. The values found in the roots and stems oscillate within the interval of the critical toxic content of the plants and approach the minimum threshold of the critical toxic content in the leaves. A slight copper contamination is recorded, particularly in the stems.

✓ Cadmium

Unlike the other heavy metals analyzed, cadmium is evenly distributed in all the organs.

Furthermore, all the cadmium values obtained are higher than the normal content of plants and lower than the critical toxicity content of plants. So the cadmium is within the standards.

There is thus a very marked "organ" effect. However, some chemical elements show a similar behavior whether at the level of diffusion in plants or their migration in tissues. This similarity is referred to as synergistic or antagonistic relationships. In our case, this similarity in behavior was observed for zinc and lead.

IV.CONCLUSION

In this research work we studied *Marrubium vulgare L*, this plant widely used in traditional Algerian pharmacopoeia for its therapeutic virtues. Who has provided access to detailed knowledge on the fate of metal pollutants (Pb, Zn, Cr, Cu and Cd) in this plant.

The phytochemical examination made it possible to characterize the flavonoids, tannins, saponosides, coumarins and reducing compounds in the three parts of our studied plants (leaves, stems and roots).

The chemical extraction of heavy metals by aqua regia in the different organs of *Marrubium vulgare L* showed a contamination by chromium in a mondre measure by copper particularly in the stems. This observation poses a major problem relating to contamination in the food chain.

Unlike other metals zinc, lead and cadmium are within the standards.

This interesting result represents a novelty and is reported for the first time in this species. Finally, this work is placed in a multidisciplinary research problem, associating analytical chemistry and biology. It opens up new perspectives in the enhancement of our flora.

Faced with these qualities of a tolerant plant and hyperaccumulator of heavy metals, we recommend cultivating Marrubium vulgare L in contaminated soils to clean them up.

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