

Chemical Composition and Antioxidant Activity of Aqueous Extracts of Some Wild Medicinal Plants in Southern Tunisia

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Abstract- This study concerned four plants known for their use in traditional medicine in south-eastern Tunisia (*Rosmarinus officinalis*, *Thymus capitatus*, *Artemisia herba-alba* and *Artemisia campestris*) whose leaves are commonly consumed as tea. We aimed to determine the chemical composition and the antioxidant activity of these aqueous extracts prepared in accordance with the traditional method (50g/L of water). Because of the containment of flavonoids, polyphenols, tannins and saponins, all aqueous extracts have electrical conductivity (total salts) which ranged from 1.12 for the *R. officinalis* extract to 4.82 mS/cm for *A. herba-alba*. The last one is the richest species in potassium (575 mg/L) and especially sodium (400 mg/L). The content of total phenolics varied from 77.7 GAE mg/L of *T. capitatus* extract to 306.35 mg GAE/L of *A. campestris* extract. The antioxidant activities expressed as IC₅₀ values varied from 3.17 μ L extract/mL of DPPH solution for the extract of *A. campestris* (the most active) to 14.33 μ L extract/mL for the *R. officinalis* extract (the least active). Given these results, we can say that the consumption of these extracts could have preventive and curative benefits against several diseases. People suffering from stomach problems or high blood pressure should show great concerns to it because of the acidity and relatively high sodium content of these extracts.

Keywords- *Rosmarinus Officinalis*; *Thymus Capitatus*; *Artemisia Herba-alba*; *Artemisia Campestris*; Antioxidant Activity; Minerals; Polyphenols; Infusion

I. INTRODUCTION

Rosemary (*R. officinalis*), sagebrush (*A. herba-alba*), rustic wormwood (*A. campestris*) and thyme (*T. capitatus*) are aromatic plants naturally grown in mountain areas of south-eastern Tunisia (Tataouine, Matmatata, Beni Khedache). They are known for their use in traditional medicine against some respiratory diseases (influenza, bronchitis, sinusitis, sore throat) and to relieve chronic diseases such as arthritis. They are also cultivated in gardens as ornamental plants and used to flavour some dishes. The fresh or dried leaves of these species are commonly consumed as infusion or decoction for treating these diseases at three coffee cups per day (about 250 to 500 mL of extract per day). Several studies concerning the chemical composition and the biological activities of these species revealed the presence of essential oils, flavonoids, tannins, saponins, and polyphenols.

Rosemary used as a food-flavoring agent, is known medicinally for its powerful extract effects of leaves such as, antibacterial (Del Campo et al., 2000), hepatoprotective (Sotelo-Félix et al., 2002), antithrombotic (Yamamoto et al., 2005), antiulcerogenic (Dias et al., 2000), diuretic (Haloui et al., 2007), antidiabetic (Bakirel et al., 2008), antioxidant

(Peng, Yuan, Liu, & Ye, 2005; Bakirel et al., 2008), antinociceptive (González-Trujano et al., 2007) and anti-inflammatory (Altinier et al., 2007). An ethnopharmacological use of *R. officinalis* in the treatment of depression, among other uses, was reported (Heinrich et al., 2006).

The sagebrush aerial part is widely used in the traditional medicine to treat diabetes, bronchitis, diarrhea and neuralgias (Tahraoui et al., 2007). The essential oil of this species was known by its therapeutic disinfectant, anthelmintic and antispasmodic virtues (Houmani et al., 2004). The antibacterial and the antispasmodic activities of *A. herba-alba* essential oil from various chemotypes have been examined (Yashphie et al., 1987; Mighri et al., 2010). The oil exhibited also antileishmaniose activity (Hatimi et al., 2001), spasmolytic activity (Perfumi et al., 1999), and antimutagenic activity against carcinogen benzopyrene (Neffati et al., 2008).

The preparation of leaves and flowers of rustic wormwood (infusion, maceration and decoction) have been recommended in Tunisian folk medicine for their antivenin (Memmi et al., 2007), anti-inflammatory, antirheumatic and antimicrobial activities (Behmanesh et al., 2007). A number of flavanones (pinostrobin, pinocembrin, sakuranetin and naringenin), dihydroflavonol (7-methyl aromadendrin) and flavone (hispidulin) have been isolated from this plant (Hurabielle et al., 1982). The hypoglycemic and hypolipidemic effects of *A. campestris* leaf extracts that due probably to its antioxidative potential in rat pancreatic tissue was reported (Mediha et al., 2010).

The thyme has traditionally been considered as an anthelmintic, antispasmodic, carminative, emmenagogue, expectorant, rubefacient, sedative, stimulant, and tonic. The plant has been used as a folk medicine against asthma, arteriosclerosis, colic, bronchitis, coughs, diarrhea, and rheumatism (Akrou et al., 2010). Thyme oil has demonstrated potent spasmolytic as well as antioxidant and antibacterial activities and is ranked among the most active oils due to the presence of cravacrol and/or thymol (Bhaskara et al., 1998, Miguel et al., 2004; Sacchetti et al., 2005; Bel Hadj Salah et al., 2006; Bouhdid et al., 2006).

In the south-eastern area of Tunisia, these species are commonly consumed as an infusion against several diseases which led us in this work to study the physicochemical characteristics of this form of preparation (infusion) to try to explain the benefits and adverse effects of this consumption and to prevent the customers in some few side effects that may cause these infusions.

II. MATERIALS AND METHODS

A. Plant Material

The aerial part was collected just before the flowering period in the region B énikhdache (southern Tunisia). After drying (room temperature), the big stems were thrown and the rest of samples (leaves and floral buds) were used for analysis.

B. Extracts Preparation

Plant materials (5g) were placed in a beaker and 100 mL of boiling distilled water were added. After 15 min, the mixture was filtered and the filtrate was used for analysis.

C. Analysis

1) Secondary Metabolites

The search for secondary metabolites (tannins, flavonoids, saponins, anthraquinones, alkaloids and essential oils) was performed according to the basic methods of the characterization tests: tannins with a ferric chloride solution; the alkaloid with Bouchardat, Mayer and Dragendorff reagents; flavonoids with magnesium fragments and chlorhydric acid; saponins by observation of the foam after shaking; anthraquinones with a chloroform and ammonia solution and essential oils by hydrodistillation with Clavenger apparatus. All tests were done in triplicate.

2) Minerals (Na and K), pH and Electrical Conductivity

Sodium and potassium of plant extracts were determined directly by using a flame photometer (SHERWOOD 410). The pH and the electrical conductivity were measured directly with a pH meter and conductivity meter (inoLab WTW) respectively.

All analyses were done in triplicate and the results were expressed as means $\pm$ SD.

3) Antioxidant Activity

We used the procedure adopted by Wong et al. (2006) with some modifications. Different concentrations of extracts and standard (ascorbic acid) were prepared by diluting an appropriate amount of distilled water. 50 μ L of each prepared solution were mixed with 2 mL of methanol and DPPH solution (4 mg of DPPH in 100 mL of methanol). After stirring with a vortex mixer, the mixture is placed at room temperature and in darkness for 30 min. Then, the measurement absorbance at 517 nm was performed after adjusting the zero absorbance with methanol by using a spectrophotometer (UV/VIS SHIMADZU brand UVmin 1240). The absorbance A_0 of control solution consisting of 50 μ L of distilled water and 2 mL of methanol solution of DPPH was measured under the same conditions.

The antioxidant activity of the standard (ascorbic acid) or extracts was expressed as the concentration of the extract or the standard providing 50% inhibition (IC_{50}). This concentration was determined graphically by plotting the curve showing the percentage inhibition against the extract concentration (in μ g plant material/mL of DPPH solution). The percentage of inhibition (% I) was calculated using the following formula:

$$\% I = 100 \times [(A_0 - A) / A_0]$$

Where A_0 is the absorbance of the control solution and A is the absorbance of sample solution or standard.

This analyse was done in triplicate and the results were expressed as means $\pm$ SD.

4) Total Polyphenols Content

An acquisition of 100 μ L of the diluted extract was placed in the presence of 2 mL of a solution of sodium carbonate to 2% and then the mixture was stirred with a vortex and let stand for 2 minutes. Then 100 μ L of an aqueous solution of 50% Folin-Ciocalteu were added. The mixture was stirred again with the vortex and kept at rest in the darkness at room temperature (18-22 $^{\circ}$ C/ 30 mn. Finally, reading the absorbance was performed with a wavelength of 760 nm and a spectrophotometer UV / VIS SHIMADZU brand UVmin 1240 after setting the zero with a mixture containing the same reagents (the sample was replaced by the distilled water). The levels of polyphenols are determined graphically from a standard curve of gallic acid representing the change in absorbance measured under the same conditions as the extracts, according to a range of concentrations of gallic acid in the prepared distilled water (Chen et al, 2007). The results are expressed in mg gallic acid equivalent per-liter of extract (EAG mg/L extract) or in mg gallic acid equivalent per-gram of plant material (EAG mg/g plant material).

This analyse was done in triplicate and the results were expressed as means $\pm$ SD.

III. RESULTS AND DISCUSSION

A. Mineral Contents

The results of the analyses were established to give nutrient values per 50g of used portion of dried weight (Table1). Mineral elements were found to vary widely depending on the different species. Sodium Contents (mg/L) were higher in A.herba-alba (400 mg/L), in similar range for T. Capitatus and A. campestris (72.1 and 80.5 mg/L, respectively) and lower in R. officinalis (7.0 mg/L). This last one is also less concentrated in potassium (46.5 mg/L) for which the T. capitatus shows a close value of 79.5 mg/L in this element. A. herba-alba and A. campestris are the richest ones in potassium (575.0 and 416.0 mg/L, respectively).

For comparison purpose, the relative knowledge of the mineral contents of wild plant species or that cultivated in the arid zone is still fragmentary. Difference might be due to growth conditions, genetic factors, geographical variations and analytical procedures (Guil et al., 1998; Ozcan and Akgul, 1998).

Throughout the world, there is an increasing interest in the importance of dietary minerals to prevent diseases. Major minerals are those required in amounts greater than 100 mgs per day and they represent 1% or less of bodyweight. Some results of mineral contents of plant species are consumed as tea, and how significant values for the Artemisia genus (A. campestris and A. herba-alba) in potassium and sodium which could reduce individual risk factors, including those related to cardiovascular disease (Anke et al., 1984; Mertz, 1982; Sanchez-Castillo et al., 1998).

B. PH and Conductivity

Containing flavonoids, polyphenols, tannins and saponins, all extracts had an electrical conductivity (total salts) which ranged from 1.12 for the R. officinalis extract to 4.82 mS/cm for A. herba-alba. All aqueous extracts of these plants are slightly acidic with a pH ranging from 4.6 to 5.9. However, it is known that both properties depend on sample dilution, due to the changes of the physical and chemical environment of the ions and to their interactions with different components present in the system such as sugars, salts and amino acid (Bell and Labuza, 1992a and Bell and Labuza, 1992b).

TABLE1. MINERAL CONTENT, PH, CONDUCTIVITY, TOTAL POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS OF THYMUS CAPITATUS, ARTEMISIA HERBA-ALBA, ARTEMISIA CAMPESTRIS AND ROSMARINUS OFFICINALIS

	pH	Na	k	Conductivity	Total polyphenols	IC ₅₀
		(mg/L)	(mg/L)	(mS/cm)	mg EAG/L	μL extract/mL
T. capitatus	5.4 ± 0.2	72.1 ± 5.1	79.5 ± 6.0	2.26 ± 0.03	77.7 ± 6.0	9.14 ± 0.70
A. herba-alba	4.6 ± 0.2	400.0 ± 20.0	575.0 ± 30.0	4.82 ± 0.03	113.6 ± 8.0	3.93 ± 0.12
A. campestris	5.2 ± 0.2	80.5 ± 6.0	416.0 ± 22.0	3.11 ± 0.02	306.7 ± 15.0	3.17 ± 0.10
R. officinalis	5.9 ± 0.2	7.0 ± 0.3	46.5 ± 3.1	1.12 ± 0.01	85.6 ± 6.5	14.33 ± 0.85

IC₅₀ of Ascorbic acid = 2.5 ± 0.2 μg/mL.

C. Total Polyphenols Content and Antioxidant Activity

The content of total phenolics expressed as GAE (gallic acid equivalent) in mg/L of plant extract, varied from 77.7 GAE mg/L of T. capitatus extract to 306.35 mg GAE/L of A. campestris extract. The phenolic compounds have been reported to be significantly associated with the antioxidant activity of plant and food extracts mainly because of their redox properties, allowing them to act as reducing agents, hydrogen donors, singlet oxygen quenchers, hydroxyl radical quenchers, and metal chelators (Gupta and Prakash, 2009). The DPPH radical is stable and free. It is commonly used as a substrate to evaluate in vitro antioxidant activity of extracts of fruits, vegetables and medicinal plants. Antioxidants can scavenge the radical by hydrogen donation, which causes a decrease of DPPH absorbance at 517 nm. The concentration of sample at which the inhibition percentage reaches 50% is defined as the IC₅₀ value. Thus, IC₅₀ value is negatively related to the antioxidant activity, the lower IC₅₀ value indicates higher antioxidant activity of the tested sample. IC₅₀ values varied from 3.17 μL extract/mL of DPPH solution for the extract of A. campestris (the most active) to 14.33 μL extract/mL for the R. officinalis extract (the least active).

The Artemisia genus shows a high content of phenolic components with strong antioxidant activity contributing to the strong antioxidant potency of the plant extracts.

IV. CONCLUSION

In southern Tunisia, The consumption of these species as infusion (herbal tea) was found to be good sources of minerals and total polyphenols and it demonstrates a strong antioxidant potency of the Artemisia genus. But people suffering from stomach problems or high blood pressure should be careful because of the acidity and relatively high sodium content of some of these extracts such as A. herba alba.

Because consumption of commercial herbal infusions is on the rise, these results give privilege to start intensive and detailed analytical studies for isolation and identification of phenolic compounds and the essential oils extracted for local species used as infusion in industrial research center. A set of complementary tests are needed to improve the complete nutrient profile and biological activities of these extract.

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