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Antioxidant activity, phenolic and flavonoid contents of some wild medicinal plants in southeastern Algeria

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This study reported on five plants known for their use in traditional medicine in southeastern Algeria, namely: *Artemisia campestris, Asteriscus pygmaeus, Pituranthos chlorantus, Pallenis spinosa* and *Aizoon hispanicum.* The work aimed to determine the antioxidant activity, phenol and flavonoid contents of their aqueous and methanolic extracts. Flavonoids and phenol contents varied according to the nature of the extract and the nature of the plant. Total phenols varied from 30.33 ± 2.03 µg EAG/mg of plant in aqueous extract of *Aizoon hispanicum* to 280 ± 5.46 µg EAG/mg plant extract in methanolic extract of *A. campestris*. Flavonoid contents were between 0.071 ± 0.0008 µg QE/mg extract in *A. hispanicum* aqueous extract and 29.68 ± 0.32 µg QE/mg extract in *A. campestris* methanolic extract. The aqueous extracts showed the lowest values of flavonoid contents while the methanol extracts showed the highest ones. The antioxidant activities expressed as IC_{50} values varied from 8.66±1.52 µg/ml for *Artemisia* aqueous extract, the most active to 325.7±5.50 µg/mL of DPPH solution to the less active *Aizoon* aqueous extract. The radical scavenging activity decreased in the following order: *A. campestris > P. spinosa> P. chlorantus > A. pygmaeus > A. hispanicum*.

Key words: Antioxidant, flavonoids, phenols, plants.

INTRODUCTION

Free radicals play a major part in the development of chronic and degenerative ailments such as cancer, autoimmune disorders, rheumatoid arthritis, cataract, aging, cardiovascular, neurodegenerative diseases and diabetes mellitus (Willcox et al., 2004; Pham-Huy et al., 2008). Oxidation process is one of the most important means for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to non-radical forms, and function as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (Halliwell, 1994; Kuhnan, 1976; Kumpulainen and Salonen, 1999; Younes, 1981).

Recently, more attention has been given to medicinal plants of therapeutic potentials as antioxidants in reducing free radical induced tissue injury. Many plants have been investigated in the search for novel antioxidants (Bol'shakova et al., 1998; Erdemoglu et al., 2006). The synthetic antioxidants have restriction for use, as they are suspected to be carcinogenic. Therefore, the

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importance of searching for and exploiting natural antioxidants has increased greatly in present years (Mervat et al., 2009).

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro et al., 2000). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli and Windell, 2009).

The genus Artemisia, widespread over the world, growing wild over the Northern Hemisphere belongs to the Asteraceae family. Eleven species of Artemisia can be found in Algerian flora (Quezel and Santa, 1963; Salido et al., 2004). *Artemisia campestris* L., known in Algeria as “dgouft” grows wild on the steppe and desert (Dob et al., 2005). In Arab folk medicine, *Artemisia campestris* L. has been used as Stomach and liver diseases Hypoglycemic, (Hammiche et al., 2006). cholagogue, choleretic, digestive, depurative, antilithiasic, obesity and cholesterol (Sijelmassi, 1993; Hmamouchi, 1999).

The genre Pituranthos has over twenty species, some of which are specific to North Africa (Quezel and Sanata, 1963; Kaabeche, 1990) and are often encountered in arid or desert regions. The species *Pituranthos chlorantus* in folk medicine is generally used as Fever, diabetes asthma, rheumatism (Hammiche et al., 2006; Vérité et al., 2004).

The genre Pallenis is a typical Mediterranean type, occurrence in the desert and coastal habitats southern Europe, northern Africa, the Canary Islands and the Middle East (Ozenda, 1991; Quezel and Santa, 1963). *Pallenis spinosa* used in folk medicine for treat Eczema, anti Rheumatism, muscular contraction, tire, vomiting for the new one born, diabetes, headaches, disinfecting (Bouabdelli et al., 2012).

The species *Asteriscus pygmaeus* is an annual plant. It is a species recognized Sahara-Sindian but also encountered in the Arabian Desert (Meyers, 1888) According Bellakhdar, the infusion of this plant is mainly used in the Sahara, for calming the stomach pain (Bellakhdar, 1997).

Aizoon hispanicum is a taxonomically isolated species, distributed in southern Mediterranean habitats from SE Spain, N Africa and S Italy to Crete (Pignatti, 1982; Greuter et al., 1984; Gonçalves, 1990). This plant is used in veterinary medicine for stimulate the milk.

The purpose of the present study was to investigate the antioxidant activity, phenol and flavonoid content of some wild plants, representative of different types of soil were collected from different regions of the southeastern algeria (Table 1).

MATERIALS AND METHODS

The aerial parts of *P. chloranthus* Benth and Hook. (Apiaceae), *A. hispanicum* L. (Aizoaceae), *A. campestris* L. (Asteraceae), *P. spinosa* (L.) Cass. (Asteraceae) and *A. pygmaeus* (DC.) Coss. and Dsev. (Asteraceae) were collected just before the flowering period in Barika region (southeastern Algeria) and identified by Dr. Sarri Djamel of M'sila university. Plant materials were dried at room temperature and powdered.

Extracts preparation

Aqueous extracts (AqE)

200 g of each powdered plant were infused in 2 L boiling distilled water set aside for 30 min and filtered. After filtration, extracts were concentrated under vacuum below 40°C and the extracts were freeze-dried.

Methanol extracts (MeE)

20 g of each plant powder was extracted in 200 ml of methanol by maceration (48 h). The solvent was removed under the vacuum at temperature below 40°C.

Determination of total phenol content

Phenolic contents were determined by Folin-Ciocalteau method (Chen et al., 2007); an acquisition of 100 µL of the diluted extract was placed in the presence of 2 ml of a solution of sodium carbonate (2%) and then, the mixture was stirred with a vortex and let to stand for 2 min. Then 100 µL of an aqueous solution of 50% Folin-Ciocalteu (Merck Co. (Germany)) was added. The mixture was stirred again with the vortex and kept at rest in the darkness at room temperature (22-25°C/30 min). Finally, reading the absorbance was performed with a wavelength of 760 nm with a spectrophotometer UV/VIS SHIMADZU 1700. The levels of total phenolic were determined graphically from a standard curve of gallic acid (Merck Co. Germany).) 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. The results are expressed in µg gallic acid equivalent per-mg of plant extract (µg EAG /mg plant extract).

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination (Bahorun et al., 1996). Each plant extracts (1 ml of 1:1 $mg.ml^{-1}$) in methanol for the MeE, and in water for AqE were separately mixed with 1 ml of 2% aluminum chloride. They remained at room temperature for 10 min. The absorbance of the reaction mixture was measured at 430 nm with a spectrophotometer (UV/VIS SHIMADZU 1700). The calibration curve was determined by preparing quercetin (Sigma Chemical Co. (St., Louis, USA).) solutions at concentrations of 0-35 µg/ml in methanol. The concentrations of flavonoids in the test samples were calculated from the calibration plot and expressed as µg Quercetin equivalent/mg of extract.

Antioxidant activity

The antioxidant activity was determined on the basis of their scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. DPPH is a stable nitrogen-centered free radical, the color of which changes from violet to yellow upon

Table 1. Ethnobotanical data of the investigated wild vegetal species of the regions of the southeastern Algeria.

Scientific name	Local name	Used parts	Preparation	Ethnomedical use	References
Artemisia campestris (Asteraceae)	Dgouft	Aerial parts, leaves, flowers	Infusion Decoction	Stomach and liver diseases; Hypoglycemic, cholagogue, choleretic, digestive, depurative, antilithiasic, obesity and cholesterol	Hammiche et al. (2006), Sijelmassi (1993), Hmamouchi (1999)
Pituranthos chlorantus (Apiaceae)	Guezzeh	Aerial Parts	Infusion	Fever, diabetes, asthma and rheumatism	Hammiche et al. (2006), Vérité et al. (2004)
Asteriscus pygmaeus (Asteraceae)	Rose of Jericho Nougd	Leaves	Infusion	calming the stomach pain	Bellakhdar (1997)
Pallenis spinosa (Asteraceae)	Nougd	Leaves	Infusion	Eczema, anti-rheumatism, muscular contraction, tire, vomiting for the new one born, diabetes, headaches, disinfecting	Bouabdelli et al. (2012)
Aizoon hispanicum L. *(*Aizoaceae)	Melah	Aerial parts		Leaves and stems are used raw or cooked. Can be used as a spinach substitute. Leaves have an acid flavor; they are thick and succulent with a slightly salty tang. Plant ash yields soda which is used in making soap and glass; Used in veterinary medicine for stimulate the milk	Phillips and Rix, (1995) Facciola (1990)

reduction by either the process of hydrogen- or electron donation (Dehpour et al., 2009). The procedure adopted by Wong et al. (2006) and modified by Akrout et al. (2012) was used. Different concentrations of extracts (0.125 to 5 mg/ml) and standard [ascorbic acid (20 to 100 µg/ml) (Merck Co. (Germany)) were prepared (MeE for methanol extract, and AqE for distilled water extract). 50 µL of each prepared solution were mixed with 2 mL of methanol and DPPH (Sigma Chemical Co. (St., Louis, USA)) solution (4 mg of DPPH in 100 mL of methanol) and kept for 30 min at room temperature and in darkness. Then, the measurement of absorbance at 517 nm was performed after adjusting the zero absorbance with methanol realized by using a spectrophotometer (UV/VIS SHIMADZU 1700).

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 µg plant extract/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.

RESULTS AND DISCUSSION

Total phenol and flavonoid contents

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003; Cook and Samman, 1996). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992).

Total phenol, total flavonoid content and the antioxidant activity of different plant extracts are shown in Table 1. Phenolic contents are expressed as µg gallic acid equivalent (GAE)/mg of plant extract, with reference to standard curve ($y = 0.0036x-0.029$, $R^2 = 0.985$). Flavonoid contents are reported as µg quercetin equivalent/mg of extract, with reference to standard curve ($y = 0.0501x + 0.0167$, $R^2 = 0.998$). It is observed that phenol and flavonoid contents in different plants differ.

Table 2. Total phenol, flavonoids contents and DPPH scavenging activities in the studied plant extracts.

Plant species	Flavonoid (µg Q E/mg extract)		Total phenol µg EAG /mg extract		Antioxidant activity IC_{50} (µg/ml)	
	AqE	MeE	AqE	MeE	AqE	MeE
Artemisia campestris	17.21±0.45	29.68±0.32	192.28±8.59	280.4±5.46	8.66±1.52	20.67±1.52
Pituranthos chlorantus	12.76±0.36	12.34±0.21	91.03±4.41	77.59±2.88	56.67±3.51	71.67±3.05
Asteriscus pygmaeus	3.93±0.18	7.63±0.39	72.59±4.72	40.71±3.09	40.67±2.08	74.33±4.72
Pallenis spinosa	5.45±0.21	25.43/0.11	125.71±4.96	71.59±4.44	19.67±3.05	49.33±3.21
Aizoon hispanicum L.	0.071±0.0008	0.17±0.007	30.33±2.03	40.39±3.21	325.7±5.50	292.3±7.02

IC_{50} of ascorbic acid = 1.38 ± 0.2 µg/mL.

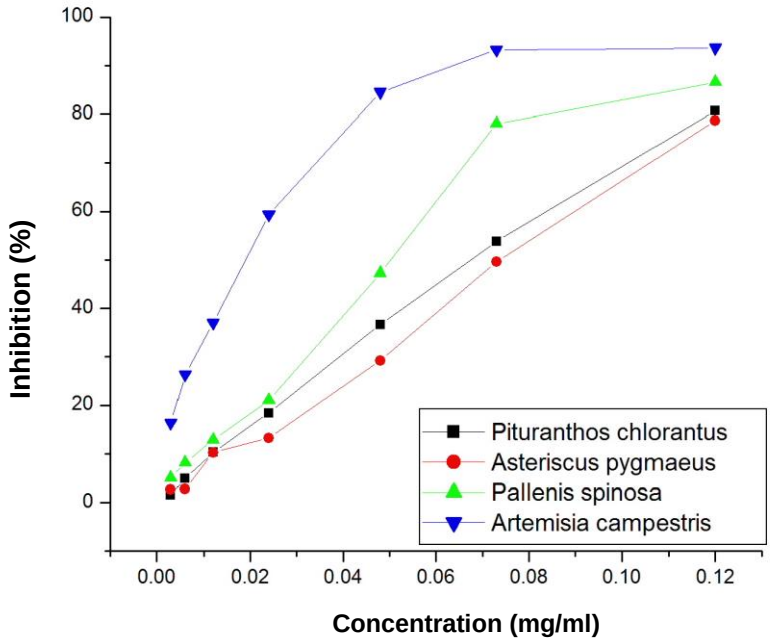

Figure 1. DPPH radical scavenging activity aqueous extract of *P. chloranthus*, *A. campestris*, *P. spinosa* and *A. pygmaeus.*

Highest phenol and flavonoid contents were noted in *A. campestris* extracts and lowest in *A. hispanicum* (Table 2).

Methanolic extracts showed the highest amount of flavonoids and aqueous extracts showed the lowest amount. This fact may be due to low solubility of these compounds in water. According to their flavonoid contents, the ranking order of the five species was as follows: *A. campestris* > *P. spinosa* > *P. chlorantus* > *A. pygmaeus* > *A. hispanicum*. Concerning phenol contents, results proved that the solvents for extraction vary individually by varying medicinal plant used, that is, total phenolic content of *A. campestris* aqueous extract was 192.28 ± 8.59 µg EAG/mg extract, while its methanol extract contained 280.4 ± 5.46 µg EAG/mg extract. Whereas, methanol extract of *P. spinosa* was 71.59± 4.44 µg EAG/mg extract of total phenolics, aqueous extract showed 125.71 ± 4.96 µg EAG/mg extract.

IC_{50} for DPPH radical-scavenging activity are shown in Table 2, and Figures 1, 2 and 3. The highest antioxidant activity was noted in the extracts of *A. campestris* plant, and the infusion of *A. hispanicum* presented a very weak or negligible activity. This result is in accordance with that reported by Akrout et al. (2011).

The general ranking of antioxidant activity decrease in the same order than phenol and flavonoid contents in each extract type. The correlation coefficient between IC_{50} data and the total phenolic compound contents is 0.81 and 0.70, for MeE and AqE respectively, confirming that these compounds are likely to contribute to the radical scavenging activity of these plant extracts. (Ivana Karabegovi et al., 2011). Lower correlation value of the

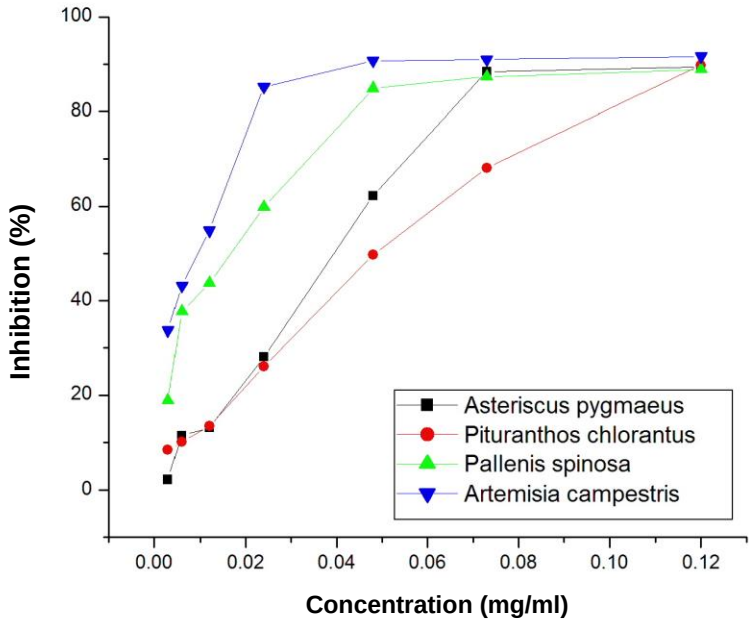

Figure 2. DPPH radical scavenging activity methanolic extract of *P. chloranthus*, *A. campestris*, *P. spinosa* and *A. pygmaeus.*

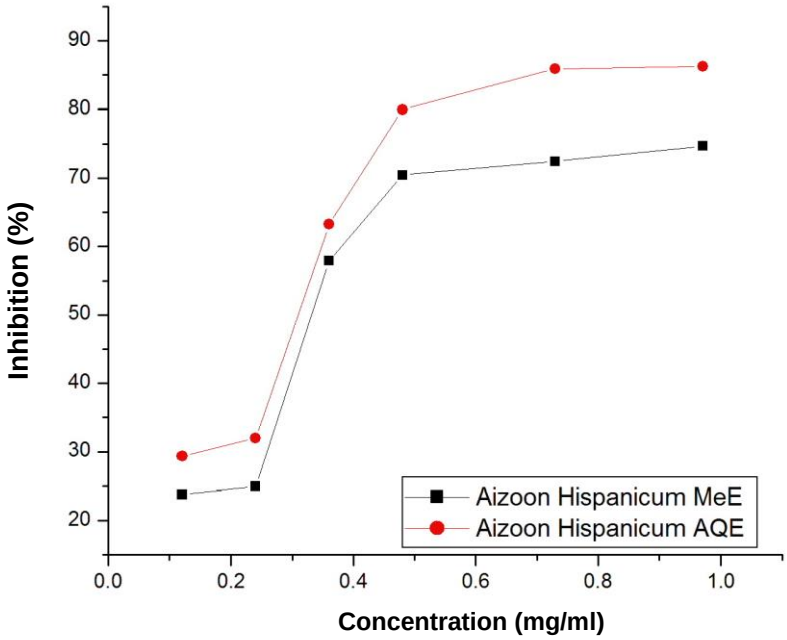

Figure 3. DPPH radical scavenging activity aqueous and methanolic extract of *A. hispanicum.*

aqueous extracts suggests that in this extracts, phenolic compounds alone are not fully responsible for the antioxidant activity of plants. A study performed by Babbar et al. (2011), showed that other water soluble constituents such as ascorbates and reducing carbohydrates as well as the synergistic effect among them could possibly contribute to the total antioxidant activity. This might indicate the relative practicability of

water as extracting solvent for the active compounds of these plants.

The implication of oxidative stress in the etiology of several chronic and degenerative diseases suggests that antioxidant therapy represents a promising avenue for treatment. In the future, a therapeutic strategy to increase the antioxidant capacity of cells may be used to fortify the long term effective treatment. The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated in situ (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). The roles of antioxidants are to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (Pham-Huy et al., 2008). In the present study all the plants except *Aizoon hispanicum* showed phenol and flavonoid content and exhibit antioxidant activity. And also their use in traditional folk medicine (Table 1). Detail work by using different methods will be the aim of further investigation.

Conclusion

The purpose of this study was to evaluate, by a chemical method, the antioxidant capacity of phenolic compounds in some Algerian plants. These plants showed significant antioxidant activity, flavonoid and phenolic contents. Among the five plants studied in this work, *A. campestris* and *P. spinosa*, both belonging to the Asteraceae family, were found to be the most promising ones. These plants contain the highest amount of phenolics and have a high level of antioxidant activity. Aqueous extract exhibited higher antioxidant activity despite its lower phenolic content. This may justify the use of plant infusion in traditional medicine.

Conflict of Interests

The authors have not declared any conflict of interests.

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