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Antioxidant activity of some algerian medicinal plants extracts containing phenolic compounds

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Abstract

Phytochemicals are extensively found at different levels in many medicinal plants. This work had two objectives: the first, to evaluate the total phenolic or flavonoid contents of 11 Algerian medicinal plants and second, to determine whether these compounds have an antioxidant capacity toward free radical propagation. The polyphenolic extractions of the dried powdered samples have been performed using 70% ethanol. The total phenolic content, analyzed using Folin–Ciocalteu's reagent, of the samples varied from 3.13 to 32.32 mg/g dry weight, expressed as gallic acid equivalents (GAE). The total flavonoid concentrations, detected using 2% aluminum chloride, varied from 1.62 to 13.12 mg rutin equivalents (RE)/g dry weight. To analyze the antioxidant activity, a common stable radical chromogen, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), was used. The antioxidant activity measurement, expressed as Trolox equivalent antioxidant capacity (TEAC), ranged from 9.40 to 33.06 mM Trolox equivalents. With further data analysis it was found that there was a positive correlation between the total phenolic content of a given sample and its antioxidant activity, $R^2 = 0.7931$, whereas the correlation between the total flavonoids and antioxidant activity was determined to be $R^2 = 0.7802$. These results suggest that the level of antioxidant activity in these plants varies to a great extent. They also suggest that phenolics in these plants provide substantial antioxidant activity. Upon achievement of this survey, and using more samples, an extra benefit of these medicinal plants may be found. Flora of Algeria appears to be a rich and interesting source for supplementary ethnomedicinal and phytochemical studies.

Keywords: Antioxidant activity; Total phenolic content; Flavonoids; Medicinal plants

1. Introduction

Herbs have been used in many domains including medicine, nutrition, flavoring, beverages, dyeing, repellents, fragrances, cosmetics, smoking, and other industrial purposes. Since the prehistoric era, herbs have been the basis for nearly all medicinal therapy until synthetic drugs were developed in the nineteenth century (Dahanukar, Kulkarni, & Rege, 2000; Exarchou et al., 2002).

The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissues (Hirasa & Takemasa, 1998). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Velioglu, Mazza, Gao, & Oomah, 1998).

Many medicinal plants contain large amounts of antioxidants such as polyphenols, which can play an important role in adsorbing and neutralizing free radi-

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cals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Anderson et al., 2001).

It has been reported that there is an inverse relationship between the antioxidative status occurrence of human diseases (Rice-Evans, Sampson, Bramley, & Holloway, 1997). In addition, antioxidant compounds which are responsible for such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders (Middleton, Kandaswami, & Theoharides, 2000; Packer, Rimbach, & Virgili, 1999). Therefore, research to identify antioxidative compounds is an important issue. Although it remains unclear which of the compounds, of medical plants are the active ones, polyphenols recently have received increasing attention because of some interesting new findings regarding their biological activities. From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial. Even though a variety of herbs are known to be sources of phenolic compounds, studies isolating polyphenols and evaluating their antioxidative effects have rarely been carried out.

The choice of our investigated plants is based on two criteria: first, in this domain there is no study in Algeria that deals with these plants, and the second criterion is that these plants have ethnopharmacological data indicating their traditional utilization in the treatment of some abdomional diseases (Table 1). In addition, they have been proved to be efficient in the treatment of various cancerous lesions of the stomach, colon, rectum esophagus and liver. Furthermore, they have been described in treating hypertention and oedema, and as detoxicant, diuretic, anti-inflammatory, anti-pyretic and anti-purulent agents. Due to their traditional utilization and active components, these plants are also considered to be efficient for the treatment of free radical-related disorders.

The purpose of this study was to evaluate a variety of 11 medicinal plants that are of the same location and have grown in the same conditions. This evaluation is related to the total phenolic content and antioxidant activity to find out new potential sources of natural antioxidants.

2. Materials and methods

2.1. Plant material

Eleven plants have been evaluated in this study, namely *Artemisia campestres* L, *Artemisia herba halba*, *Artemisia arboresens* L, *Artemisia arvensis* L, *Juniperus oxycedrus* L, *Globularria alypum* L, *Oudneya africana*, *Thymeelaea hirsuta*, *Ruta monata* L, *Thapsia garganica* and *Teucrium polium* L.

The medicinal plants were gathered in June 2002, from different places around the town of laghouat in the steppe region of Algeria. The various data (local name, medicinal uses, used parts of plant, method of preparation and administration) were collected from local inhabitants having knowledge of the curative properties of these plants.

2.2. Chemical reagents

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany).

2.3. Extraction of phenolics

The air dried plant material (1 g) was crushed and extracted for 24 h with 50 ml of 70% (v/v) aqueous ethanol

Table 1
Medicinal plants commonly used in Algerian popular medicine

Plant name	Popular medicine use		
	Digestive	Anti-inflammatory	Others
Apiaceae (*Thapsia garganica*)	*		
Asteraceae (*Artemisia campestris*)	*		
Asteraceae (*Anthemis arvensis*)	*		
Asteraceae (*Artemisia herba halba*)	*		
Compositaes (*Artemisia arboresens*)	*		
Cruciferaceae (*Oudneya africana*)	*		
Cupressaceae (*Juniperus oxycedrus*)	*		*
Globulariaceae (*Globularia alypum*)	*	*	*
Labiatae (*Teucrium polium*)	*		
Rutaceae (*Ruta montana*)	*		*
Thymeleacees (*Thymelaea hirsute*)		*	*

at room temperature. After removal of ethanol under reduced pressure in a rotary evaporator at 40 °C, the remaining aqueous solution of the extraction was defatted twice with petroleum ether to remove lipids. Then the lyophilized solution was extracted with ethyl acetate, in the presence of aqueous solution with 20% ammonium sulphate, and 2% of meta-phosphoric acid solution. The ethyl acetate fraction was dried by adding a sufficient amount of anhydrous sodium sulphate, and then evaporated to dryness using a rotary evaporator. The precipitate was dried, dissolved in 5 ml of absolute methanol and kept at −20 °C.

2.4. Analysis of total phenolic compound

The amount of total phenolics was determined with the Folin–Ciocalteu reagent using the method of (Lister & Wilson, 2001). This method was employed to evaluate the phenolic content of the samples. A standard curve must first be plotted using gallic acid as a standard. Different concentrations of gallic acid were prepared in 80% of methanol, and their absorbances were recorded at 765 nm. 100 µl of sample was dissolved in 500 µl (1/10 dilution) of the Folin–Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500 µl of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a Milton Roy 601 UV–Vis spectrophotometer and the results are expressed in mg of gallic acid per g (GEA) of dry weight of plant.

2.5. Estimation of flavonoids content

The flavonoids content in extracts was determined spectrophotometrically according to Lamaison and Carnat (Quettier-Deleu et al., 2000), using a method based on the formation of a complex flavonoid–aluminium, having the absorbtivity maximum at 430 nm. Rutin was used to make the calibration curve. 1 ml of diluted sample was separately mixed with 1 ml of 2% aluminum chloride methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with a Milton Roy 601 UV–Vis spectrophotometer and the flavonoids content was expressed in mg per g of rutin equivalent (RE).

2.6. Quantification of total antioxidant activity (ABTS assay)

The total antioxidant activity (TAA) values were estimated by the Trolox equivalent antioxidant capacity (TEAC) test (Miller & Rice-Evans, 1996). In this test, we measured the relative capacity of antioxidants to scavenge the $ABTS^{\cdot+}$ radical compared to the antioxidant potency of Trolox is used as a standard.

The $ABTS^{\cdot+}$ radical generated by mixing an ABTS 20 mM solution with 70 mM $K_2S_2O_8$ in the dark for 24 h, at room temperature. Before usage, the $ABTS^{\cdot+}$ solution was diluted to get an absorbance of 0.700 ± 0.020 at 734 nm with PBS at pH 7.4 (Phosphate buffered saline prepared by mixing 5 mM of NaH_2PO_4, 5 mM of Na_2HPO_4 and 153.84 mM of NaCl in 1 l of distilled water).

The spectrophotometer is preliminary blanked with PBS. Upon adding 2.0 ml of the diluted $ABTS^{\cdot+}$ solution to 10 µl of antioxidant sample or Trolox standard, the absorbance at 734 nm was recorded each minute after initial mixing. Appropriate solvent blanks were run in each assay, and all measurements are done at least 5 min. Falls in absorbance were noted and then calculated and plotted with respect to time and concentration of the standard and samples. The final TEAC value of the antioxidant compound was calculated by comparing $ABTS^{\cdot+}$ decolourisation with Trolox, which gives a useful indication of the antioxidant potential of the plant extracts.

2.7. Statistical analysis

The results were presented as the means ± SEM. Student's test was used to analyze the statistical significance. Correlation analysis of antioxidant activity versus the total phenolic content were carried out using the correlation and regression programme in the EXCEL program.

3. Results and discussion

3.1. Total phenolic content

The amount of total phenolics varied in different plants and ranged from 3.1 to 32.3 mg GAE/g of dry material. The highest total phenolic levels have been detected in "*Anthemis arvensis* L", "*Artemisia campestris* L" and "*Globularia alypum* L", and the lowest in "*Artemisia arboresens* L" and "*Ruta montana* L" (Table 2).

It has been noted that amount of total phenolic compounds in Aseteraceae varieties is higher then the other families. The amount of total phenolic compounds in all tested plants was higher than some Asian vegetables (Kaur & Kapoor, 2002) some herbs and medicinal plants such as *Armoracia rusticana*, *Fallopia convolvulus*, *Matricaria matricarioides*, *Trifolium hybridum* and *Typha latifolia* (Kähkönen et al., 1999). From analysis, we can deduce that all these plants are rich in flavonoids. We outline that the amount of flavonoids in the aerials plants varies from 13.20 to 1.62 mg/g rutin equivalent

Table 2
Total phenol and flavonoid contents for the studied plants mg/g dry weight

Plant extract	Total phenolic (mg GAE/g dw)	Flavonoids content (mg RE/g dw)
Anthemis arvensis	32.32 ± 0.2	13.12 ± 0.1
Artemisia campestris	20.38 ± 0.30	7.46 ± 0.20
Globularia alypum	21.54 ± 0.81	4.54 ± 0.09
Artemisia herba halba	13.06 ± 0.40	11.31 ± 0.51
Juniperus oxycedrus	12.66 ± 0.41	3.50 ± 0.50
Oudneya africana	7.75 ± 0.22	7.66 ± 0.23
Thapsia garganica	7.63 ± 0.61	4.04 ± 0.42
Thymelaea hirsuta	6.81 ± 0.40	4.95 ± 0.81
Teucrium polium L	4.92 ± 0.21	4.63 ± 0.10
Artemisia arboresens	3.42 ± 0.50	3.25 ± 0.31
Ruta montana	3.13 ± 0.30	1.62 ± 0.40

of the crude extract. However, we can state here that in such studies, an extraction produce must be remove non phenolic substances such as sugars, proteins and pigments which may interfere during the total phenolic evaluation. Upon such extraction, we have obtained results which show a significant high total amount of the phenolics. We also mention here that an increase of the phenolic metabolism in these saharian plants may be related to the hard climate conditions (hot temperatures, high solar exposure, dryness, short growing season).

According to the phenolic and flavonoid amount, Anthemis arvansis and all Artemisia species all belonging to family Asteraceae, hydroxycinnamic and hydrobenzoic derivatives predominated, as well as in *Globularia alypum* (family Globulariacea) and juniperus oxycedrus. In other, flavonoids were the main phenolic subgroup except in *Thymelea hirsuta* (family Gobulariacea) and juniperus oxycedrus. In other extracts, flavonoids were the main phenolic subgroup except in *Thymelea hirsuta* (family Thymelaeaceae) and *Oudneya africana* (family Cruciferaceae). The phenolic extracts ware evaluated for their antioxidant effectiveness in a dynamic way using a chemical method (TEAC assay).

3.2. Antioxidant activities

The presence of different antioxidant components in the plant tissues makes it relatively hard to quantify each antioxidant component separately. Therefore, in many studies, several intermediate extractions are used to ensure a maximum extraction of the available antioxidants (Kähkönen et al., 1999). The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing as agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metallic chelating potential (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995).

The ABTS assay has been calibrated with the water-soluble α-tocopherol analogue, Trolox. All plant extracts show antioxidant activities proving their capacity to scavenge the $ABTS^{\bullet+}$ radical cation. The decrease in absorbance for each extract in intervals of time is determined, and plotted (Fig. 1). All these extracts present a linear variation of the inhibition power with the added concentration of extract (Figs. 2a and 2b). The plots suggest that at lower concentrations, the relationship between concentration and the decrease in the Absorbance is linear too. However, it should be expected that at higher concentrations of the sample, the plot would reach a plateau. These results suggest that all plant extracts are good inhibitors at low concentrations. The antioxidant activity measurements of ethanolic extracts, expressed as Trolox equivalent antioxidant capacity (TEAC) are presented in Table 3.

Since TEAC is a quantification of the effective antioxidant activity of the extract, a higher TEAC would imply greater antioxidant activity of the samples. For "*Ruta*

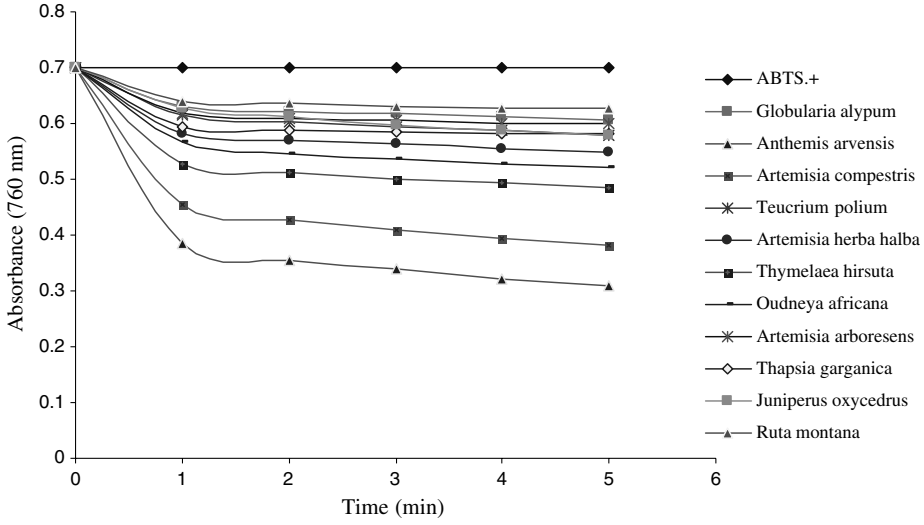

Fig. 1. Plot of absorbance fall with respect to the concentration of plant extras (1–5 min).

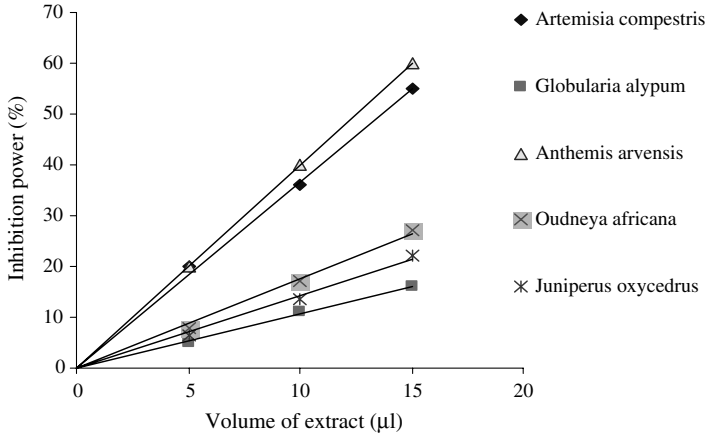

Fig. 2a. Concentration-response curves for inhibition of the absorbance of $ABTS^{\cdot+}$ cation at (734 nm) for five plant extracts.

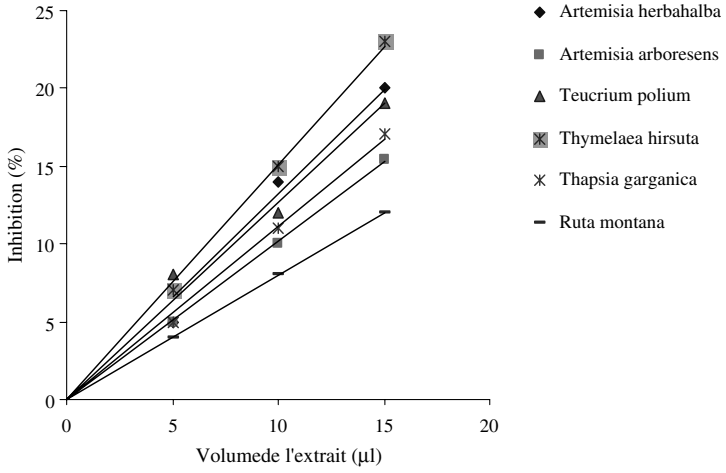

Fig. 2b. Concentration–response curves for inhibition of the absorbance of $ABTS^{\cdot+}$ cation at (734 nm) for six plant extracts.

Table 3
TEAC values of different phenolic extracts

Plant extract	Antioxidant activity (mmol TEAC/g dw)
Anthemis arvensis	33.10
Artemisia campestris	25.00
Globularia alypum	20.31
Thymelaea hirsuta	17.62
Oudneya africana	16.30
Thapsia garganica	15.30
Teucrium polium	15.00
Artemisia arboresens	13.32
Artemisia herba halba	11.60
Juniperus oxycedrus	10.70
Ruta montana	9.41

montana L" and "*Anthemis arvensis* L" the TAA varies from 9.40 to 33.06 mM Trolox equivalents, respectively (Table 3). Our results are of very good agreement with previous studies on some Lamiaceous plants (Zheng & Wang, 2001), Iranian Ocimum accessions (Javanmardi, Stushnoff, Lockeb, & Vivanco, 2003), and show equivalent or higher antioxidant activity.

Our results revealed also that the studied medicinal herbs exhibit clearly a higher antioxidant activity and contain significantly more phenolics than the common vegetables and fruits (nutritional plants). However, it appears that the TEAC values depend mainly on the assay conditions (Van den Berg, Haenen, Van den Berg, Van den Berg Vijgh, & Blast, 2000). The reason for this dependence is that the reaction of a given antioxidant with $ABTS^{\cdot+}$ does not usually reach its end point within the time lapse allowed. By comparison between the TEACs values given in different reports, one can state that the applied time interval must be considered, although different results on the time dependency in the TEAC assay have been reported in previous studies (Miller, Rice-Evans, & Papaganga, 1997; Schofield & Braganza, 1996; Van den Berg, Haenen, Van den Berg,

& Blast, 1999). In our analysis, the TEAC value is determined within 5 min and during this time laps, the slow reaction should make a significant contribution.

The correlation coefficient between TAA and total phenolic contents of Algerian plants is $R^2 = 0.79$ (Fig. 3), whereas for the correlation between the total flavonoids and antioxidant activity this coefficient is determined to be $R^2 = 0.78$ (Fig. 4). This result suggests that 79% of the antioxidant capacity of Algerian medicinal plants is due to the contribution of phenolic and flavonoids compounds. Also, it can be concluded that the antioxidant activity of plant extracts; is not the result of these compounds but may be also related to the presence of some individual active phenolic compounds.

The unclear relationship between the antioxidant activity and the total phenolics may be explained in numerous ways, in fact, the total phenolics content does not incorporate all the antioxidants. In addition, the

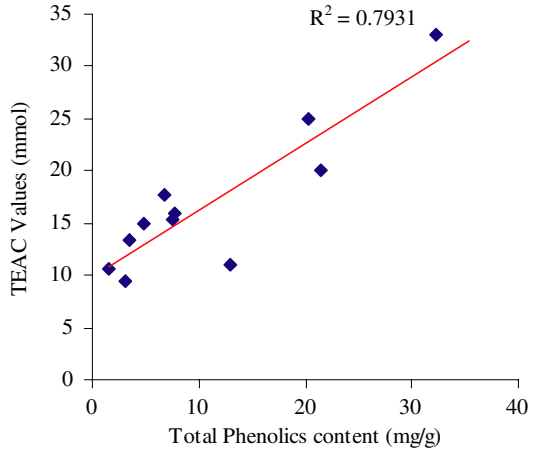

Fig. 3. Linear correlation of Trolox equivalent antioxidant capacity (TEAC) with respect to the total phenol content of 11 Algerian plants.

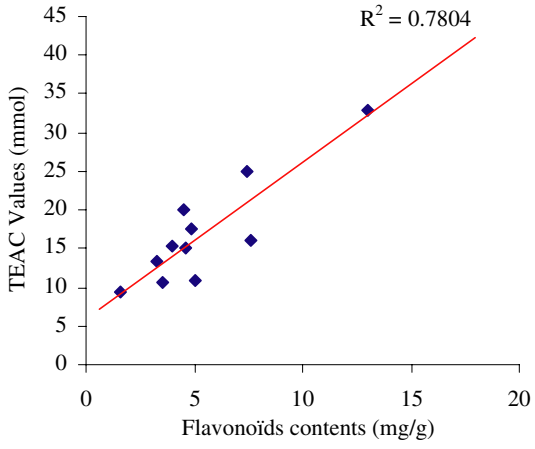

Fig. 4. Linear correlation of Trolox equivalent antioxidant capacity (TEAC) with respect to the total flavonoid content of 11 Algerian plants.

synergism between the antioxidants in the mixture makes the antioxidant activity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants. This is the reason why samples such as *Artemisia arboresens* A and *Ruta montana*, with similar concentrations of total phenolics, may vary in their antioxidant activities (TEAC assay). The results suggest that the phenolic compounds contribute significantly to the antioxidant capacity of the medicinal plants.

Several studies are focussed on the relationship between the antioxidant activity of the phenolics compounds, as hydrogen donating free radical scavengers and their chemical structure. It has been shown that the presence of the —CH=CH—COOH group in the hydroxylated cinnamates ensures greater H-donating ability and subsequent radical stabilization than the carboxylate group in the hydroxy benzoates (Rice-Evans, Miller, & Paganga, 1996). Previous studies (Castellucio et al., 1995; Chen & Ho, 1997; Heinon, Meyer, & Frenkel, 1998; Larson, 1998) showed that antioxidant activity correlated with hydroxycinnamics derivatives in nutritional plants and this corroborates with our results, although the antioxidant activity of phenolics mainly depends on the number and the position of hydrogen-donating hydroxyl groups on the aromatic cycles of the phenolic molecules (Dziedzic & Hudson, 1983; Liens, Ren, Bui, & Wang, 1999; Rice-Evans et al., 1996).

The purpose of this study was the evaluation by a chemical method of the antioxidant capacity of phenolic compounds in some Algerian medicinal plants. These medicinal plants showed stronger antioxidant activity and content in phenolics than the common nutritional plants. Among the 11 plants treated in this work, *Artemisia arvensis* and *Artemisia campestris* were found to be the most promising ones. These plants contain the highest amount of phenolics and have a very high level of Trolox equivalents.

It has been also noted in this study that these Algerian plants are strong radical scavengers and can be considered as good sources of natural antioxidants for medicinal and commercial uses. However, due to the diversity and complexity of the natural mixtures of phenolic compounds in these plant extracts, it is not easy to characterize every compound and assess their antioxidant activities. Each herb contains generally different phenolic compounds with different amount of antioxidant activity. Upon this study, we can state that in vivo studies are needed to further confirm the advantageous quality of these extracts. In several situations in popular medicine, the effect of these plants for therapeutic uses is not obvious, and the question which arises in such position is: is there any relationship between the therapeutic properties of these plants and their antioxidant properties?

In order to confirm the antioxidative effect of these promising plants, a further survey which uses other

kinds of antioxidant assays is now underway. This survey includes also the characterization of the active phenolic antioxidants.

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