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Evaluation of phenolic contents and antioxidant activities of some medicinal plants growing in Algerian Aurès Mountains

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Abstract: *The objective of this study is to evaluate the antioxidant activities of six medicinal plants growing in Algerian Aurès Mountains. Total phenolic and flavonoids contents were measured using colorimetric methods, and the antioxidant capacities were evaluated using the DPPH radical scavenging and β-carotene bleaching tests. Juniperus phoenica L. had significantly the higher total phenolic compounds (53.6±3.86 mg GAE.g^{-1} DM) ($p<0.05$); followed by Romarinus officinalis L. (26.1±3.15 mg GAE.g^{-1} DM) and Artemisia campestris L. (20.5±1.99 mg GAE.g^{-1} DM). Artemisia campestris L. had significantly the higher flavonoid contents (11.1±0.56 mg QE.g^{-1} DM) than other studied plants ($p<0.05$). The best antiradical activity was observed in Thymus algeriensis extracts ($EC50=11.1±0.33$ µg.ml^{-1}) and Romarinus officinalis L. ($EC50=15.3±0.9$ µg.ml^{-1}). β-carotene bleaching test showed that the herbs' phenolic compounds Antioxidant Activity (AA%) value was found in the range of 64-84%, whereas that of the standard antioxidant ascorbic acid was 51±2.4%. The present results indicate that medicinal plants from the Algerian Aurès mountains could be explored in food and pharmaceutical industries for development of natural's antioxidant agents.*

Keywords: Medicinal plants, phenolic compounds, oxidative stress, antioxidant activity

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Introduction

The living organism produces free radicals in an excessive way, which conducts to the oxidative stress that would cause numerous pathological damages like cancer, cardiovascular, neurodegenerative, and autoimmune diseases. Natural antioxidants are a group of substances able to enhance the organism's defense system. Vitamins, alkaloids, terpenes, and phenolics isolated from medicinal plants were largely investigated to find natural antioxidants [1]. It is well known that antioxidants have significant inhibitory effects on various free radical species and also neutralize nonradical species such as hydrogen peroxide. Additionally, they can prevent the production of many reactive oxygen species in various diseases [2]. Synthetic antioxidants have been used antioxidants. Currently, the possible toxicity of these synthetic antioxidants has been criticized. Many researchers have focused on the search for natural compounds with antioxidant properties because of the harmful effects of synthetic antioxidants in the human body [3].

The use of herbal medicine is on the increase globally. In Africa, over 80% of the population depend directly on plants for their primary healthcare requirements [4]. Commonly known, natural products from medicinal plants represent one of the most important raw materials used for treating various human diseases because of the supreme availability of chemical diversity. With multiple biological activities, many plants contain antioxidant activity which attracts the attention of several research teams for its role in the fight against numerous illness and numerous studies have identified compound within herbal plants that are effective antibiotics [5].

The medicinal plants contain significantly higher levels of phenolic compounds than common vegetables, and fruits and also exhibit stronger antioxidant activity. Phenolics are one of the most groups of phytochemicals studied in recent times due to their various and high biological activities. Phenolic acids, flavonoids, stilbens, coumarins, tanins, lignans and more of other phenolic compounds have been found to exhibit properties as anticarcinogenic, anti-hypertensive, antiproliferative, anti-inflammatory, antimicrobial, and antioxidants [1]. The presence of antioxidants in plants can provide protection against a number of diseases; for example, ingestion of natural antioxidants has been inversely associated with mortality and morbidity from degenerative disorders. Medicinal plants are therefore being investigated for their antioxidant activity, and the demand for natural antioxidants. Flavonoids and other phenolics have been suggested to play a preventive role in the development some diseases [6]. In this context, the aim of this work is to determine phenolic compounds, flavonoid contents, DPPH radical scavenging and β-carotene bleaching test of methanolic extracts of six medicinal plants, widely used in the Algerian Aurès Mountains for their therapeutic properties.

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Material and Methods

The medicinal plants choice and sampling

The plants chosen were *Artemisia herba alba* Asso, *Artemisia campestris* L., *Thymus algeriensis* Boiss. and Reut., *Juniperus phoenica* L., *Marrubium vulgare* L. *Rosmarinus officinalis* L., and Teucrium polium. These plants were chosen because they are widely used in the Aurès Mountains in the treatment of several diseases (Table 1). Plant material was collected between March and April 2015, in Aurès Mountains (Department of Batna, Algeria) (Figure 1). The plants were confirmed and authenticated by Dr. Nassima Diab, a botanist at the Technical Institute of Agricultural Development Saharan Africa (ITDAS) province of Biskra, Algeria.

Table 1. Summary of information on the different selected medicinal plants and medicinal use from Algerian region studies

Scientific name	Common name	Local name	Medicinal use from Algerian region studies
Artemisia herba alba Asso	White wormwood	*elchih, elchiha, izry*	Analgesic, antispasmodic, blood purification, cancer, carminative, diabetes, diarrhea, eczema, genital diseases, menstrual pains, respiratory system diseases, stomachache, vermifuge, ulcer [7-11]
Artemisia campestris L.	Wormwood	*tegouft, dkoufeth, tagoufed*	antispasmodic, diabetes, weight loss, antidote, wound, kidneys, antihypertensive, goiter, cough, bronchitis, Intestinal bloating, intestinal parasites, digestive diseases, fever [7, 8, 10-12]
Juniperus phoenica L.	Phoenician juniper	*elaraar, zinba, ifezi*	Urinary system diseases, respiratory system diseases, hypoglycemic, migraine, rheumatism, articular system diseases, dermatological diseases, eye infections, genital infections, gastric ulcer, vertigo, stomach pain, gases, gastrointestinal complaints, thyroid [7, 8, 11]
Marrubium vulgare L.	Common white	*jaida, imezrith, timezourin*	Analgesic, antispasmodic, cardiovascular, fever, migraine, respiratory system diseases, system diseases, tonic, wound, digestive disorders, leishmanicidal, arthritis, diabetes, fever [7, 8, 10, 11, 13]
Rosmarinus officinalis L.	Rosemary	*iklil eldjebel, klil, azri, touzala*	anemia, antidepressive, asthma, cholesterol, memory, teeth, antihypertensive, antitumoral, hepatic diseases, diabetes, stomachache, Hypotensive, wart, eczema, menstrual problems,

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			hypercholesterolemia [7-10, 13]
Thymus algeriensis Boiss. and Reut.	Thyme	*jertil, mezzouchen, z'aitra*	Toothache, gingivitis, mouth ulcers, muguet [11]
Teucrium polium L.	Cat tyme, Hulwort	*elmerrouyth, ifezi, temerrouyth*	Coagulant, stomachache, diabetes, digestive diseases, fever, gases, arterial, hypertension, women infertility, wounds [7, 9, 10, 12]

Before extracting and analyzing bioactive compounds from plants, samples must be collected, stored and prepared properly. To avoid degradation of the bioactive compounds, the samples are dried. Indeed, the high moisture or water content contributes to enzymatic activities. Before drying, the plants were cleared of insects from the sand and all other kinds of contamination. The plants were spread out in a homogeneous manner and they were dried in the open air (10 days on average for the different plants) in a dry, ventilated place and protected from light at ambient temperature (about 25°C). Since heating and exposure to light and oxygen can affect the phenolic composition in many cases. Having become dry, the leaves were separated from the stems and subdivided into small fragments, they were collected in clean plastic boxes for later use in extraction and different types of analysis.

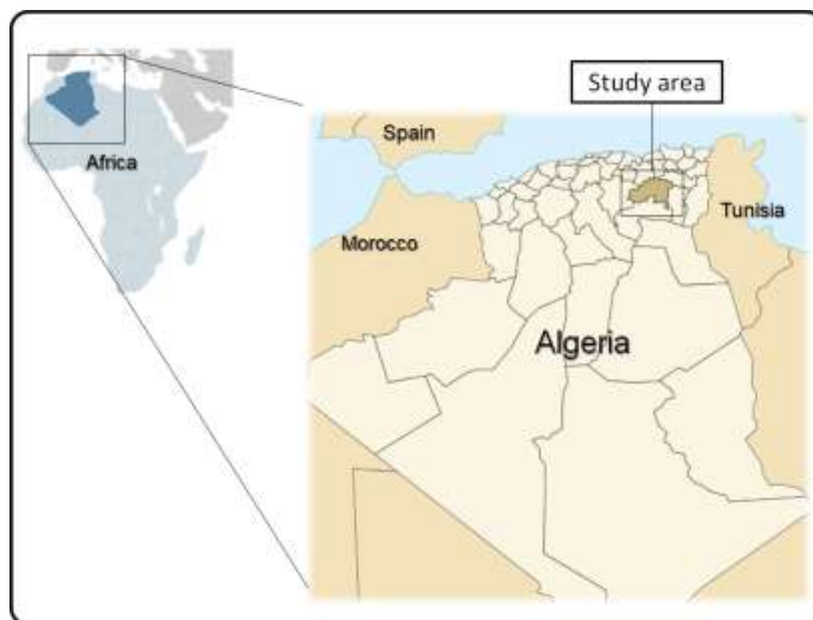

Figure 1. Geographical location of the study area

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Extraction of crude phenolic compounds

Total phenolic compounds, were extracted as described by Falleh et al. [14] with some modifications: 3 g of the plant powder (Grinder, China) was mixed with 30 ml absolute MeOH (Sigma-Aldrich, Germany) with magnetic stirring (Snijders, Holland) for 30 min, allowed to rest at 4°C for 24 hours, filtered twice on filter paper and stored at 4°C until use.

Determination of total phenolic compounds

The determination of the total phenolic compounds of the plant extracts was carried out with the Folin-Ciocalteu colorimetric reagent, according to the method of Singleton et al. [15]. Four hundred microliters (400 µl) of each extract (dissolved in methanol) was added to 2 ml of Folin-Ciocalteu reagent (Sigma-Aldrich, USA) which was already diluted 10 times. The two elements were mixed and incubated for 4 minutes. After incubation, 1.6 ml sodium carbonate solution Na2CO3 (Panreac, MW=106) (0.075 $g.ml^{-1}$) was added to get the final mixture which was stirred and incubated again in a water bath at 30°C for 30 minutes. The absorbance of the extracts was measured at 765 nm using a spectrophotometer (Shimadzu, Japan). The results were expressed in milligrams gallic acid equivalent per gram of dry matter (mg $GAE.g^{-1}$ DM).

Determination of total flavonoids

The quantification of total flavonoids was performed by a method based on the formation of complexes between flavonoids and aluminum trichloride (AlCl3) [16]. Equal volumes (1 ml) of plant extract and AlCl3 solution (2%) were mixed. Their absorbance was measured at 415 nm, after incubation at ambient temperature for 40 min. The flavonoids contents of the plant extract were expressed in milligrams quercetin equivalent per gram of dry matter (mg $QE.g^{-1}$ DM).

DPPH radical scavenging assay

The antiradical activity of phenolic extracts of the various plants was measured by the method of Dung et al. [17]. One hundred microliters (100 µl) of different dilutions of phenolic extracts were mixed with 1 ml of a 0.004% (w/v) methanolic solution of DPPH (1,1-diphenyl-2-picrylhydrazyl) in dry cuvettes. After 30 min incubation at ambient temperature, the absorbance was measured at 517 nm. A negative control was composed 100 µl of methanol and 1ml of the DPPH solution. Positive control of Trolox ($C_{14}H_{18}O_4$) was measured under the same conditions as the test sample. The antiradical activity is expressed as a function of the reducing power of the DPPH methanol solution [18], applying the following formula:

RP (%)=[(AC – AE)/AC] x 100.

Where, RP: reducing power in percentage; AE: absorbance of the DPPH solution in the presence of the phenolic extracts and Trolox; AC: absorbance of the DPPH solution in the absence of phenolic extracts or trolox.

The variations of the reducing power as a function of the concentration of the phenolic extract allow

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us to calculate the EC50 parameter. The EC50 "Efficient Concentration" is defined as the concentration of the antioxidant necessary to reduce 50% of free radicals in the reaction medium. More EC50 values are low over the anti-radical activity is high and vice versa [19]. The average EC50 values were calculated by linear regressions of the three separate tests, where the abscissa is represented by the concentration of the compounds tested and the ordinate by the antioxidant activity expressed as a percentage.

β-carotene bleaching test

The antioxidant activity of phenolic extracts of the various plants was carried out by the β-carotene bleaching method [20]. Ten milligrams (10 mg) of β-carotene were dissolved in 100 ml chloroform. One milliliter (1 ml) of this solution was added to a boiling flask containing 20 mg linoleic acid and 100 mg Tween 20. Chloroform was removed by evaporation at 50°C for 5 min by a rotary evaporator. Next, 50 ml of oxygenated distilled water was slowly added to the residue to form the emulsion (A). In open-capped cuvettes, 5 ml of the emulsion (A) was mixed with two hundred microliters (200 µl) of phenolic extract of each plant, at a concentration of 4000 micrograms per milliliter of methanol. A negative control consisting of 200 µl of methanol and 5ml of emulsion (A) was prepared without phenolic extracts. A second emulsion (B) consisting of 20 mg linoleic acid, 100 mg Tween 20 and 50 ml oxygenated distilled water was also prepared. A blanc solution of 200 µl of methanol and 5 ml of the emulsion (B) was prepared to be used as a zero value. The cuvettes were placed in a water bath at 50°C and the oxidation of the emulsion was monitored spectrophotometrically (Shimadzu, Japan) at 470 nm after 120 min. ascorbic acid (Sigma, USA) and was used as a positive control, the same protocol was applied as that phenolic extract. The antioxidant activity was expressed as a percentage and calculated by the following equation described by Ismail et al. [21]:

$$AA (\%)=[1 - (AE' – AEx) / (AC' – ACx)] \times 100.$$

Where, AA (%): antioxidant activity; AE': value of absorbance of the emulsion in the presence of phenolic extract or ascorbic acid measured at t=0 min; AC': value of absorbance of the emulsion of negative control measured at t=0 min; AEx: value of absorbance of the emulsion in the presence of phenolic extract or ascorbic acid measured at t=120 min; ACx: value of absorbance of the emulsion of negative control measured at t=120 min.

Data analysis

Phenolic compounds, flavonoid and antioxidant results were expressed as means±standard deviations (SD). Statistical analysis for all tests was carried out using ANOVA test followed by the Tukey's Honest Significant Differences post hoc test. Statistica software version 10 (StatSoft. Inc., Tulsa, OK, U.S.A.) was used to perform the statistical analysis at the 5% significance level.

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Results

Total phenolic contents

The total phenolic contents of medicinal plants measured using the Folin–Ciocalteu method are shown in Table 2. The values ranged from 8.33 to 53.65 mg GAE.g^{-1} DM. *Juniperus phoenica* L. showed the highest level (53.65±3.86 mg GAE.g^{-1} DM) ($p<0.05$); followed by *Rosmarinus officinalis* L. (26.07±3.15 mg GAE.g-1 DM), *Artemisia campestris* L. (20.53±1.99 mg GAE.g^{-1} DM) and *Teucrium polium* L. (15.61±0.43 mg GAE.g-1 DM).

Table 2. Total phenolic contents of methanolic extracts of the studied plants

Plant species	Total phenolics (mg GAE.g^{-1} DM)
Artemisia herba alba Asso	8.64±1.42^{a}
Artemisia campestris L.	20.53±1.99^{bc}
Juniperus phoenica L.	53.65±3.86^{d}
Rosmarinus officinalis L.	26.07±3.15^{c}
Thymus algeriensis Boiss. and Reut.	8.33±1.15^{a}
Teucrium polium L.	15.61±0.43^{ab}

Values are given as mean±standard deviation (SD) (n=3). Values followed by the different letters are significantly different at the 5% level ($P<0.05$).

Flavonoid contents

The total flavonoids contents of medicinal plants were measured using the Aluminum Trichloride method. According Djeridane et al. [22] flavonoids are the main phenolic group in most botanical families. In our case most of the plants tested had a high quantity of flavonoids as shown in Table 3. The amount of total flavonoids ranged from 2.95 to 11.11 mg QE.g^{-1} DM. *Artemisia campestris* L. (11.11±0.56 mg QE.g^{-1} DM) had significantly higher flavonoid contents than other studied plants ($p<0.05$).

Table 3. Total flavonoids contents of methanolic extracts of the studied plants

Plant species	Total flavonoids (mg QE.g^{-1} DM)
Artemisia herba alba Asso	5.47±1.06^{a}
Artemisia campestris L.	11.11±0.56^{b}

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Juniperus phoenica L.	5.18±1.31[a]
Rosmarinus officinalis L.	5.22±1.92[a]
Thymus algeriensis Boiss. and Reut.	2.95±0.25[a]
Teucrium polium L.	2.98±0.09[a]

Values are given as mean±standard deviation (SD) (n=3). Values followed by the different letters are significantly different at the 5% level (P <0.05).

Antiradical capacity

This part is devoted to evaluation using a chemical method the antioxidant capacity phenolic compounds in selected medicinal plants. Numerous and diverse techniques are available to evaluate the antioxidant activities of specific compounds or complex mixtures such as phenolic compounds. The results of such screening may be used to select the most effective extracts for a more comprehensive examination [23]. Therefore, in the current study, two assays were conducted in order to evaluate in vitro antioxidant properties of our phenolic compound's samples: DPPH (1.1-diphenyl-2-picrylhydrazyl) radical scavenging and β-carotene bleaching test.

The values for 50% scavenging activity (EC50) are presented in Table 4. The variance analysis performed on the DPPH scavenging activity of the phenolic compounds showed significant differences among herbs (p<0.05). The different extracts herbs exhibited remarkable reduction activities. The scavenging activity EC50 value was found in the range of 1.41 to 33.71 $\mu g.ml^{-1}$. The best activity was observed in *Rosmarinus officinalis* L. (EC50=1.41±0.07 $\mu g.ml^{-1}$), *Thymus algeriensis* Boiss. and Reut. (EC50=1.60±0.13 $\mu g.ml^{-1}$), and *Artemisia campestris* L. (EC50=2.47±0.11 $\mu g.ml^{-1}$) extracts; the lowest (EC50=33.7±1.15 $\mu g.ml^{-1}$) in *Artemisia herba alba* Asso extracts. Also, the activities of all medicinal plants treated are, significantly, higher than that trolox, with EC50=46.6±1.98 $\mu g.ml^{-1}$, as standard reference product.

Table 4. Antioxidant activity of different extracts as determined by DPPH radical scavenging activity

Plant species	EC_{50} Value of phenolic compounds ($\mu g.ml^{-1}$)
Artemisia herba alba Asso	33.71±1.15[b]
Artemisia campestris L.	2.47±0.11[a]
Juniperus phoenica L.	6.30±0.35[a]
Rosmarinus officinalis L.	1.41±0.07[a]
Thymus algeriensis Boiss. and Reut.	1.60±0.13[a]

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Teucrium polium L.	5.36±0.50[a]
Trolox	46.62±1.98[c]

Values represent mean±standard deviation (SD) of three replicates. Different letters (a-f) indicate a significant difference between the antioxidant activities ($p<0.05$).

Antioxidant capacity

The β-carotene bleaching test method is based on the loss of the yellow colour of β -carotene due to its reaction with radicals, which are formed by linoleic acid oxidation in an emulsion. The antioxidant activity of the treated herbs' phenolic compounds was examined by comparing it to the activity of known antioxidants such as ascorbic acid. All results are reported in Table 5. These results showed that the herbs' phenolic compounds antioxidant activity (AA%) value was found in the range of 64.3% to 84.5%, whereas that of the standard antioxidant ascorbic acid was 51.2±2.4%. Consequently, all treated herbs had a higher antioxidant activity than standard antioxidant following β-carotene bleaching test. *Rosmarinus officinalis* L. (84.54±2.71%) and *Artemisia campestris* L., (80.08±3.41%) extracts were slightly better than the extracts of *Juniperus phoenica* L. (75.78±2.46%); both being quite better in comparison to the extracts of *Artemisia herba alba* Asso, *Thymus algeriensis* Boiss. and Reut., and *Teucrium polium* L. (67.56±1.75%, 64.31±1.9%, and 64.84±3.1%, respectively).

Table 5. Antioxidant Activity (AA%) of different extracts as determined by β-carotene bleaching activity

Plant species	AA of Phenolic extract (%)
Artemisia campestris L.	80.08±3.41[cd]
Artemisia herba alba Asso	67.56±1.75[bc]
Juniperus phoenica L.	75.78±2.46[bcd]
Rosmarinus officinalis L.	84.54±2.71[d]
Thymus algeriensis Boiss. and Reut.	64.31±1.9[ab]
Teucrium polium L.	64.84±3.1[ab]
Ascorbic acid	51.24±2.36[a]

Values represent mean±standard deviation (SD) of three replicates. Different letters (a-d) indicate a significant difference between the antioxidant activities ($p<0.05$). AA%: Antioxidant Activity.

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Discussion

Total phenolic compounds

The value of total phenolic contents of medicinal plants measured using the Folin–Ciocalteu method ranged from 8.33 to 53.65 mg GAE.g^{-1} DM. In present study, *Juniperus phoenica* L. have the highest total phenolic compounds; followed by *Rosmarinus officinalis* L., *Artemisia campestris* L., and *Teucrium polium* L. It is well known that plant phenolic compounds are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations [24]. Thus, the increase of the phenolic compounds in arid and semi-arid area plants may be related to the hard climate conditions (high solar exposure, hot temperatures, short growing season, dryness etc.) [22].

The total phenolic compounds of *Juniperus phoenica* L. was 53.65±3.86 mg GAE.g^{-1} DM. Our results aren't in agreement with those Hayouni et al. [25], the amount of total phenolic compounds of *Juniperus phoenica* L. growing in Tunisia ranged from 89.6 to 167 mg GAE.g^{-1} DM (extraction with different solvents).

The total phenolic compounds of *Rosmarinus officinalis* L. were 26.07±3.15 mg GAE.g^{-1} DM. Our results aren't in agreement also with those cited in literature. The amount of total phenolic compounds of *Rosmarinus officinalis* L. growing in Morocco [26] and in Turkey [27] was 185.71±4 mg GAE.g^{-1} DM and 119 mg GAE.g^{-1} DM, respectively.

The total phenolic compounds of *Artemisia campestris* L. and *Artemisia herba alba* Asso were 20.53±1.99 and 8.64±1.42 mg GAE.g^{-1} DM, respectively. Our results are in agreement with those Djeridane et al. [22], the amount of total phenolic compounds of *Artemisia campestris* L. and *Artemisia herba alba* Asso growing in Algeria were 20.38±0.30 mg GAE.g^{-1} DM and 13.06±0.40 mg GAE.g^{-1} DM respectively. However, the amount of total phenolic components in the different extracts of *Artemisia herba alba* Asso growing in Tunisia was 123.95±4.30 g GAE.kg^{-1} DM [28].

Hayouni et al. [25] have been showed that phenolic compounds content is strongly dependent on the solvents. This investigation has been showed that a higher content of phenolic compounds was obtained with an increase in the polarity of the solvent used. The qualitative and quantitative variability of secondary metabolisms in medicinal plant species screened in the current study can be related to several factors mainly: stage of plant growth, genetic pool, the state of plant material, geographical and environmental conditions including soil composition, climatic conditions with emphasis on stressful environmental conditions such as drought of semi-arid and arid zones, seasonal variations, and soils characteristics [29, 30].

Total flavonoid contents

The total flavonoids contents of medicinal plants were measured using the Aluminum Trichloride method. In our case, all plants tested had a high quantity of flavonoids. The total flavonoids of *Artemisia campestris* L. and *Artemisia herba alba* Asso were 11.11±0.56 and 5.47±1.06 mg QE.g^{-1} DM, respectively.

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Our results are in agreement also with those Djeridane et al. [22], the amount of total flavonoids of *Artemisia campestris* L. and *Artemisia herba alba* Asso growing in Algeria were 7.46±0.20 mg QE.g^{-1} DM and 11.31±0.51 mg QE.g^{-1} DM, respectively. Nevertheless, the total flavonoids content of an infusion of *Artemisia campestris* L. growing in Tunisia have been determined by Megdiche-Ksouri et al. [30] 175.23±7.26 mg QE.g^{-1} DW and Ghlissi et al. [31] 28.30±1.45 mg RE.g^{-1} of extract (Rutin Equivalent), these amounts are higher than found in our investigation. According to Da Silva Port's et al. [32], comparison of total phenolic content with the literature data is difficult, since just a few studies were previously carried out with the same species and even when the species were the same, they originated from other regions. Another problem is the use of different reference standards and different extraction solvents.

Antiradical activity

This part is devoted to evaluation using a chemical method the antioxidant capacity of phenolic compounds in some Algerian medicinal plants. Numerous and diverse techniques are available to evaluate the antioxidant activities of specific compounds or complex mixtures such as phenolic compounds. The results of such screening may be used to select the most effective extracts for a more comprehensive examination. None of these methods on its own is ideal for evaluating antioxidant activity but together; they nonetheless provide useful information on extracts and individual compounds [23, 33]. Therefore, in the current study, two assays were conducted in order to evaluate *in vitro* antioxidant properties of our phenolic compounds samples: DPPH radical scavenging and β-carotene bleaching test.

The method is based on the reduction of DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm appearing in a deep violet colour. In DPPH test the antioxidants react with the stable free radical 1.1-diphenyl-2-picrylhydrazyl (deep violet colour) and convert it to 1.1-diphenyl-2-picrylhydrazine with discoloration. The degree of discoloration indicates the free radical scavenging potentials of the sample/antioxidant [20, 34]. The results are highly reproducible and comparable to other free radical scavenging methods [24].

The variance analysis performed on the DPPH scavenging activity of the phenolic compounds showed significant differences among herbs ($p<0.05$). The activities of all medicinal plants treated were higher than that trolox as standard reference product. According Cai et al. [35] the large variation in the antioxidant activity of medicinal plants is due to the diversity of phenolic compounds, their concentrations and the different molecular structural characteristics of these compounds. Li et al. [36] found a correlation between antioxidant capacities and the total phenolic content of 45 medicinal plants. Therefore, a high content of phenolic compounds is an important factor in determining the antioxidant capacities of medicinal plants. The best activity was observed in *Rosmarinus officinalis* L. (EC_{50}=1.41±0.07 µg.ml^{-1}), *Thymus algeriensis* Boiss. and Reut. (EC_{50}=1.60±0.13 µg.ml^{-1}), and *Artemisia campestris* L. (EC_{50}=2.47±0.11 µg.ml^{-1}) extracts.

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According to the literature, the antioxidants activities of *Rosmarinus officinalis* L. are mainly linked to the phenolic compounds present in the rosemary extract. The most active being phenolic acids. Carnosic acid is the major phenolic constituent present in rosemary leaves, with an antioxidant activity approximately 7 times greater than that of synthetic antioxidants [37].

Antioxidant activity

Phenolic compounds are complex of mixture with different functional groups, polarity and chemical behaviours, and this could lead to scattered results, depending on the test employed. Therefore, the use of multiple methods is necessary in the assessment of antioxidant activity [38]. Consequently, the β-carotene bleaching method was also used. Our results are in concordance with several studies that demonstrated the antioxidant action of *Artemisia campestris* L. extracts following β-carotene bleaching test. Ethyl acetate extract produced 82% of bleaching inhibition and exceeded that of BHT; similarly, chloroform extract also inhibited 79% of β-carotene bleaching [39]. Same results were observed (84% and 88.7%) of β-carotene bleaching [31, 40], respectively. Antioxidant activity of compounds with different structures can be compared using the β-carotene bleaching, because these methods are based on the inhibition of linoleic acid oxidation reactions. The DPPH method measures only a compound's reaction with DPPH which is dependent on its structural conformation; thus, comparisons may not always be appropriate [41].

Conclusions

As a conclusion, the extracts of the six medicinal plants showed free radical (DPPH) scavenging and strong inhibition of linoleic acid activities when compared to standards such as trolox and ascorbic acid, respectively. The results of this study showed that the extract of medicinal plants growing in Aurès mountains can be used as easily accessible source of natural antioxidants and in food and pharmaceutical industry. Finally, the findings of this work are useful to further research such as the identification of specific phenolic compounds responsible for the antioxidant activities of *Artemisia campestris* L., *Juniperus phoenica* L., *Rosmarinus officinalis* L., and *Thymus algeriensis* Boiss. and Reut.

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