Food Chemistry 112 (2009) 303–309

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Antioxidant capacity and phenol content of selected Algerian medicinal plants

Dina Atmani, Nassima Chaher, Meriem Berboucha, Karima Ayouni, Hakima Lounis, Hania Boudaoud, Nadjet Debbache, Djebbar Atmani *

Laboratory of Applied Biochemistry, Faculty of Life and Nature Sciences, University of Bejaia, Bejaia 06000, Algeria

ARTICLE INFO

Article history:
Received 16 January 2008
Received in revised form 29 April 2008
Accepted 20 May 2008

Keywords:
Pistacia lentiscus
Fraxinus angustifolia
Clematis flammula
Aqueous extracts
Phenols
Algeria
Lipid peoxidation
DPPH radical

ABSTRACT

Extracts from the bark of *Fraxinus angustifolia* as well as the leaves of *Pistacia lentiscus* and *Clematis flammula* have been investigated for their reducing power, inhibition of linoleic acid peroxidation and scavenging capacity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and H_2O_2 using in vitro spectrophotometric methods. The results indicate that the best overall antioxidant capacity was shown by extracts of *Pistacia lentiscus*, followed by *Fraxinus angustifolia* and *Clematis flammula*.

Pistacia lentiscus aqueous fraction obtained from chloroform partition showed a high and dose-dependant reducing power (IC50 = 50.03 μg/ml), a very high scavenging ability against DPPH radical (IC50 = 4.24 μg/ml) and an outstanding activity against linoleic acid peroxidation (IC50 = 0.82 μg/ml). *Fraxinus angustifolia* aqueous fraction issued from chloroform extraction also exhibited a high DPPH scavenging activity (IC50 = 10.0 μg/ml) and a high activity against linoleic acid peroxidation (IC50=5.06 μg/ml). In contrast, it is the organic (chloroform) fraction of *Clematis flammula* that showed a high activity against linoleic acid peroxidation (IC50 4.6 μg/ml), whereas the aqueous fraction of chloroform partition showed a moderate scavenging activity against DPPH (IC50 = 25.02 μg/ml). The IC50 values for the antioxidant of reference, butylated hydroxyanisole (BHA) are 5.19, 6.16 and 71.16 μg/ml for inhibition of linoleic acid peroxidation, DPPH scavenging activity and reducing power, respectively. With the exception of *Pistacia lentiscus* aqueous extracts obtained from hexane and chloroform partitions that showed high H_2O_2 scavenging ability, all the extracts from *Fraxinus angustifolia* and *Clematis flammula* showed only moderate and even weak capacity, respectively.

The observed results which correlate positively with total phenol content strongly plead in favour of the use of these plants as potential food additives in replacement of synthetic compounds.

1. Introduction

The human body produces reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and hydrogen peroxide by many enzymatic systems through oxygen consumption. In small amounts, these ROS can be beneficial as signal transducers (Finkel, 1998) and growth regulators (Hancock, Desikan, & Neill, 2001). However, during oxidative stress, large amounts of these ROS can be produced and may be dangerous because of their ability to attack numerous molecules, including proteins and lipids (Halliwell, Gutteridge, & Cross, 1992). In fact, it has been reported that ROS largely contribute to cellular aging (Sastre, Pallardo, & Vina, 2000), mutagenesis (Takabe et al., 2001), and coronary heart disease (Khan & Baseer, 2000) through several ways, including membrane destabilisation (Mora, Paya, Rios, & Alcaraz, 1990), DNA breakage (Takabe et al., 2001) and generally by oxidising low-density lipoproteins (LDL). The cell can reduce the impact of ROS either by an endogenous system implicating enzymes such as catalase and superoxide dismutase or by an exogenous system using antioxidants, vitamin C and α-tocopherol (Cheesman & Slater, 1993).

It has been suggested that the intake of fruits and vegetables is associated with a low risk of cancer and cardiovascular disease (Knekt, Jârvinen, Reunanen, & Maatela, 1996). A great number of plants worldwide showed a strong antioxidant activity (Baratto et al., 2003; Katalynic, Milos, Kulisic, & Jukic, 2006) and a powerful scavenger activity against free radicals (Kumaran & Karunakaran, 2007; Vellosa et al., 2006). This antioxidant capacity can be explored in food industry by using plants as a source of antioxidants to prevent the rancidity and oxidation of lipids. In fact, in recent years, research has focused on medicinal plants to extract natural and low-cost antioxidants that can replace synthetic additives such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) that might be carcinogenic (Whysner, Wang, Zang, Iatropoulos, & Williams, 1994) and even toxic (Moure et al., 2001). Furthermore, a lot of herbs are used as spices and have also

* Corresponding author. Tel.: +213 34214513; fax: +213 34214762.
E-mail address: djatmani@yahoo.com (D. Atmani).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.077

anti-microbial activities that prevent the growth of food-borne pathogens (Hirasa & Takemasa, 1998).

The beneficial health effects of plants are attributed to flavonoids, a class of secondary metabolites which protect the plant against ultraviolet light and even herbivores (Harborne & Williams, 2000). Using a variety of experimental model systems, it has been found that the protective effects of flavonoids are due to their capacity to transfer electrons to free radicals and to chelate metal catalysts (Ferrali et al., 1997), activate antioxidant enzymes (Elliot, Scheiber, Thomas, & Pardini, 1992), reduce α-tocopherol radicals (Hancock et al., 2001) and inhibit known free radical producing enzymes, such as myeloperoxidase and NADPH oxidase (Middleton & Kandaswami, 1992) and xanthine oxidase (Nagao, Seki, & Kobayashi, 1999). Furthermore, flavonoids have demonstrated exceptional cardioprotective effects, essentially because of their capacity to inhibit LDL peroxidation (Mazur, Bayle, Lab, Rock, & Rayssiguier, 1999).

Ethnopharmacological data obtained from herbalists indicate that *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* are extensively used in folk medicine by rural populations in Algeria. The leaves of *Pistacia lentiscus* purify water and increase the time of conservation of dry figs and sun-dried tomatoes; they are also used as appetiser and astringent. The bark of *Fraxinus angustifolia* is best known for its use as an anti-inflammatory; however, it is also used as antioxidant, diuretic, digestive and astringent. *Clematis flammula* leaves are used to treat arthritis and superficial burns. They are also used as an insect repellent to prevent deterioration of stored wheat and corn. However, so far, there are no studies on the antioxidant potential of these plants. In this study, we examined the antioxidant capacity of extracts from these plants using several tests: reducing power, scavenging capacity against the radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and H_2O_2 and inhibition of linoleic acid peroxidation.

2. Materials and methods

2.1. Materials

The leaves of *Pistacia lentiscus* and *Clematis flammula* and the barks of *Fraxinus angustifolia* were harvested in remote areas in the suburbs of the city of Bejaia located in Northeastern Algeria and dried away from direct sunlight. Plants were identified at the laboratory of botany (University of Bejaia) where specimens have been deposited. Dried plant material was then ground to fine powder (diameter < 63 μm) using an electric mill (Kika Labortechnik, Staufen, Germany). All chemicals were purchased from Sigma (represented by Algerian Chemical Society, Setif, Algeria).

2.2. Extraction procedure

The extraction of active compounds from plant material was carried out using the method developed by Chiang, Lo, and Lu (1993), with minor modifications. Plant powder was macerated in ethanol (1:4, w/v) during 24 h. The mixture was centrifuged at 1500 g/min and the supernatant was recovered and dried to yield the crude ethanol extract which was partitioned into ethyl acetate and water (1:3:1, w/v/v). After a maceration of 24 h, two distinct phases were obtained; these were collected and dried separately to give two different extracts: the organic and the aqueous. The organic extract was divided into two equal parts and further partitioned into (i) hexane and water and (ii) chloroform and water (1:3:1, w/v/v). The resulting phases were separated and dried giving rise to two organic extracts and two aqueous extracts. At the end, seven different extracts for each plant were collected and tested for various activities.

2.3. Reducing power

The reducing power of extracts from *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* was determined spectrophotometrically (SPECORD 50) according to the protocol of Oyaizu (1986). The plant extract (1.0 ml) was added to 2.5 ml of phosphate buffer (0.2 M, pH6.6) and 2.5 ml of potassium ferrocyanate [$K_3Fe(CN)_6$] (1%). After incubation at 50 °C for 20 min, 2.5 ml of trichloroacetic acid (10%) were added to the mixture before centrifugation at 88*g* over 10 min. The supernatant was gathered and mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). The absorbance was calculated at 700 nm and compared to two standards, BHA and α-tocopherol; any increase in absorbance is synonymous of an increase in reducing power.

2.4. Inhibition of linoleic acid peroxidation

The antioxidant activity of extracts from *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* was determined spectrophotometrically according to the thiocyanate method of Osawa and Namiki (1981) with minor modifications. Plant extracts (2.0 ml) were mixed with 2.05 ml of linoleic acid (2.51%) in pure ethanol, 4.0 ml of phosphate buffer (0.05 M, pH 7.0) and 1.95 ml of distilled water and incubated at 40 °C. A blank solution was prepared the same way as above except that plant extract was replaced with ethanol. Every 24 h, a fraction of this mixture (50 μl) was taken and transferred to a tube subsequently supplemented with 4.85 ml ethanol (75%) and 50 μl ammonium thiocyanate. The absorbance was recorded at 500 nm 3 min after addition of 50 μl of ferrous chloride (0.02 M) prepared in hydrochloric acid (3.5%). This procedure was repeated until the absorbance of the blank has reached its maximal value (96 h). % inhibition of peroxidation = 100-[(increase in absorbance of extract/increase in absorbance of control) 100].

2.5. Scavenging activity against the diphenyl-picrylhydrazyl (DPPH) radical

The capacity of extracts from *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* to reduce the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed using the method of Masuda et al. (1999). Fifty microliters of a solution of DPPH in methanol (5 mM) were mixed with 2.45 ml of a solution of plant extract during 30 min and the absorbance was recorded at 517 nm. The normal purple color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. The scavenging activity of extracts was evaluated according to the formula: percent scavenging = $[A_0\text{-}(A_1\text{–}A_S)]/A_0]100$, where A_0 is the absorbance of DPPH alone, A_1 is the absorbance of DPPH + extract and A_S is the absorbance of the extract only.

2.6. Scavenging activity against hydrogen peroxide

The scavenging capacity of *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* extracts on hydrogen peroxide was determined according to the method of Ruch, Cheng, and Klaunig (1989). Test tubes were prepared with 2.0 ml of various plant extracts and a solution of H_2O_2 (1.2 ml, 40 mM) in phosphate buffer (pH 7.4). A blank solution was prepared the same way but without H_2O_2. After incubation of the mixture during 10 min, the absorbance was recorded at 230 nm. The scavenging activity was calculated using the following formula: % scavenging activity = $[(A_c\text{–}A_t)/A_c]$ 100, where A_c is the absorbance of the control and A_t is the absorbance of the extract.

2.7. Determination of total phenols, flavonoids and tannins

Determination of total phenols in the extracts of the leaves of *Pistacia lentiscus* and *Clematis flammula* and the barks of *Fraxinus angustifolia* was carried out using the method of Lowman and Box (1983). The reaction mixture was obtained with 2.5 ml of a solution of plant extract (0.1 mg/ml) boiled in methanol, 25 ml of distilled water, 1.5 ml of sodium carbonate Na_2CO_3 (200 g/l) and 0.5 ml (1 N) of Folin–Ciocalteu's reagent. After incubation for 60 min at room temperature, the absorbance is recorded at 750 nm. A standard curve is prepared with the same procedure using catechin and the concentration of total phenols is expressed in mg of catechin equivalent per gram of powder.

Determination of the flavonoid content was achieved using the method described by Maksimovic, Malencié, and Kovacevié (2005) by addition of aluminum chloride reagent to the solution containing the extract. The absorbance was read at 430 nm and concentrations of flavonoids were deduced from a standard curve and calculated in mg quercetin equivalent.

Tannins were estimated according to the protocol developed by Hagerman and Butler (1978) on the basis of their precipitation by a protein, bovine serum albumin (BSA). The method is based upon the obtention of a coloured complex Fe^{++}-phenols which can be measured spectrophotometrically at 510 nm. Concentrations of tannins in various extracts are obtained in mg of tannic acid equivalent from a standard curve. For the crude extract, the amounts are expressed as mg equivalent/g of powder, whereas in the case of subsequent fractions, the amounts are expressed as mg equivalent/g of previous extract.

2.8. Statistical analysis

All assays were carried out in triplicates and results are expressed as mean ± SD. Statistical comparisons were done with the Anova test. Differences were considered to be significant at $p < 0.05$ or $p < 0.01$. The IC_{50} values were calculated using the Graph Pad Prism 4 software.

3. Results and discussion

3.1. Reducing power

The reducing power of various extracts (100 μg/ml) of the three plants are presented in Fig. 1 where we can see that the best reducing power is obtained from the aqueous fractions issued from hexane (0.91 ± 0.03) and chloroform (0.99 ± 0.01) of *Pistacia lentiscus*, significantly higher ($p < 0.01$) than that of the standards, BHA and α-tocopherol, respectively (0.68 ± 0.006 and 0.23 ± 0.01). The reducing power of *Fraxinus angustifolia* extracts at the same concentration is generally poor, except that of the aqueous extracts issued from ethyl acetate and chloroform which are 0.28 ± 0.006 and 0.28 ± 0.005, respectively, comparable to that of α-tocopherol (0.23 ± 0.014). The reducing power of *Clematis flammula* extracts was the poorest; the highest being that of the aqueous extract issued from chloroform (0.13 ± 0.0017).

The aqueous fractions issued from hexane and chloroform (partitions) of *Pistacia lentiscus* that exhibited high reducing power at 100 μg/ml were further tested at lower concentrations. Half inhibitory concentrations (IC_{50}) of these extracts are 50.03 μg/ml and 50.10 μg/ml, respectively, significantly lower than those of the standards, BHA (71.2 μg/ml) and α-tocopherol (226.4 μg/ml).

Recent studies have shown that the reducing power of methanol extracts of *Pistacia terebinthus*, a plant extensively used in Turkish folk medicine to treat burns, asthma, bronchitis and other respiratory problems, is even higher than that of α-tocopherol (Topçu et al., 2007). The latter finding strongly supports our data since the leaves of Algerian *Pistacia lentiscus* are also widely used throughout the country to treat the same disorders.

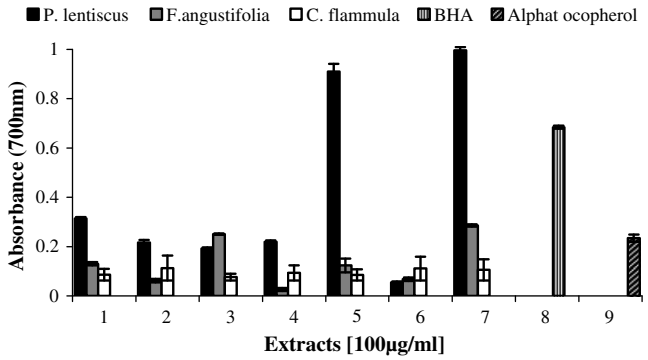

Fig. 1. Reducing power of extracts from *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* at 100 μg/ml (1. Ethanol, 2. Ethyl acetate, 3. Aqueous/ethyl acetate, 4. Hexane, 5. Aqueous/hexane, 6. Chloroform, 7. Aqueous/chloroform, 8. BHA and 9. Alpha tocopherol).

3.2. DPPH scavenging activity

The scavenging activity of extracts from the three plants against the stable radical (DPPH) has been evaluated at a concentration of 100 μg/ml and the results are illustrated in Fig. 2. Concerning *Pistacia lentiscus*, apart from the chloroform extract, all others showed high scavenging activity (90%), equivalent to that of the standard, BHA (89%). In the case of *Fraxinus angustifolia*, only three extracts exhibited high scavenging activity: the ethanol and the aqueous fractions from ethyl acetate and chloroform extractions with 78 ± 0.93, 90.29 ± 0.29 and 89.48 ± 0.33%, respectively. As to *Clematis flammula,* apart from the aqueous fraction of chloroform which showed high scavenging activity (89.9%), comparable to that of BHA and the aqueous fraction of hexane which showed a scavenging activity higher than 50% (69.26%), all the other extracts exhibited activities lower than 50%.

Further studies using varying concentrations (10–100 μg/ml) of extracts from the three plants were also carried out. In the case of *Pistacia lentiscus*, our results indicate that the aqueous extracts issued from chloroform and hexane partitions that exhibited the best reducing power also showed outstanding DPPH scavenging activity (IC_{50} = 4.24 μg/ml and 4.51 μg/ml, respectively), significantly lower than that of BHA (6.18 μg/ml). Our results are supported by the fact that methanol extracts from Turkish *Pistacia terebinthus* exhibited a DPPH scavenging activity of more than 90% at 100 μg/ml (Topçu et al., 2007).

Concerning *Fraxinus angustifolia*, the best scavenging activities were shown by the aqueous extracts issued from ethyl acetate and chloroform extractions (IC_{50} = 10.0 μg/ml and IC_{50} = 10.04 μg/ml), respectively. Nevertheless, the above results indicate that this plant, widely distributed in Algeria, exhibited better scavenging activities than closely-related plants used in other studies. Indeed, in a study using a large variety of medicinal plants, Han, Lo, Choi, Kim, and Back (2004) found that the methanol extract of *Fraxinus rhyncophylla* showed a scavenging activity against DPPH of only 50% at a concentration of 75 μg/ml. Another investigation involving three medicinal plants showed that the DPPH scavenging activity of the methanol extract of *Fraxinus excelsior* is higher than that of hexane and dichloromethane extracts, suggesting that the hydrogen-donating compounds are more likely to be present in polar solvents (Middleton et al., 2005). On the other hand, even though the aqueous fraction issued from chloroform of *Clematis flammula* showed a scavenging activity that is almost as high as

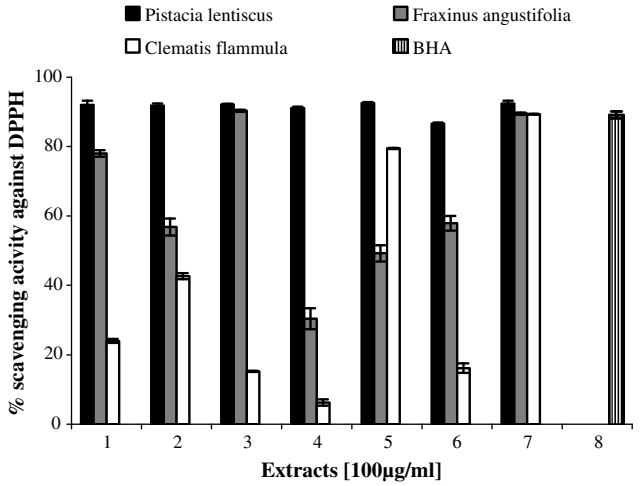

Fig. 2. Scavenging activity of extracts from *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* against the radical DPPH at a concentration of 100 µg/ml (1. Ethanol, 2. Ethyl acetate, 3. Aqueous/ethyl acetate, 4. Hexane, 5. Aqueous/hexane, 6. Chloroform, 7. Aqueous/chloroform, 8. BHA).

that of BHA at 100 µg/ml, its IC_{50} is relatively high (25.02 µg/ml), suggesting that its scavenging potential against DPPH is moderate. A study concerning *Clematis armandii*, a plant used in Chinese medicine against eczema, consolidates our results since it also showed a relatively moderate DPPH scavenging capacity (Kirby & Schmidt, 1997).

3.3. H_2O_2 scavenging activity

Fig. 3 shows that, at a concentration of 100 µg/ml, all extracts from both *Pistacia lentiscus* and *Fraxinus angustifolia* showed fairly high and moderate scavenging capacity against H_2O_2, respectively. While the scavenging activity of *Fraxinus angustifolia* extracts varies between 33.83 and 51.05%, that of *Pistacia lentiscus* extracts is between 22.5 and 75.11% and that of the controls is 28.08% for α-tocopherol and 32.98% for BHA. However, *Clematis flammula* extracts showed poor scavenging activity against H_2O_2, the highest being that of chloroform (19%). H_2O_2 scavenging activities of aqueous phases resulting from hexane and chloroform extractions of *Pistacia lentiscus* are high, strongly suggesting that these extracts contain the necessary compounds for radical elimination. In a study carried out on *Pistacia terebinthus*, it was found that the methanol leaf extract, at 50 µg/ml, had the same scavenging activity (67%) on H_2O_2 as that of ascorbic acid at 12.5 µg/ml and even better than that of butylated hydroxytoluene (BHT) (50%) at the same concentration (Topçu et al., 2007). It is well established that hydrogen peroxide is not dangerous as it is, but may well be because of its ability to form the hydroxyl radical, thereby emphasising on the importance of its elimination. Indeed, it has already been proven that dietary phenols protect mammalian and bacterial cells from cytotoxicity induced by H_2O_2 (Nakayama, 1994; Nakayama, Yamaden, Osawa, & Kawakishi, 1993), indicating that the observed H_2O_2 scavenging activity of our plants could be due to the presence of phenols.

3.4. Inhibition of linoleic acid peroxidation

Fig. 4 shows that all the extracts of *Pistacia lentiscus* as well as the aqueous extracts of *Fraxinus angustifolia* and all organic extracts of *Clematis flammula* inhibited strongly linoleic acid peroxidation. The results obtained for chloroform (99%) and ethyl acetate (97.4%) extracts of *Clematis flammula* are comparable to those of the standard, BHA (98.65%) and the two aqueous extracts of *Pistacia lentiscus* issued from chloroform (99%) and hexane (98.77%), respectively. When tested at lower concentrations, the respective IC_{50} values (0.84 µg/ml and 0.82 µg/ml) of the two aqueous extracts of *Pistacia lentiscus* are significantly ($p < 0.01$) much lower than that of BHA (5.0 µg/ml) while those of the chloroform and ethyl acetate fractions of *Clematis flammula* are 4.64 µg/ml and 4.66 µg/ml, respectively. Interestingly, the two aqueous extracts of *Fraxinus angustifolia* issued from ethyl acetate and chloroform which have shown comparable inhibition of lipid peroxidation at 100 µg/ml (93 and 94%, respectively) have nevertheless different IC_{50} values (0.45 µg/ml and 5.06 µg/ml, respectively). The results of the three plants are considered to be noteworthy when compared to the findings of other studies concerning medicinal plants in Turkey (Tepe, Sokmen, Akpulat, Yumrutas, & Sokmen, 2006) and in Pakistan (Sultana, Anwar, & Przybylski, 2007) that showed a maximum of 90% inhibition. More recently, Li and his colleagues showed that *Fraxinus rhynchophylla* was ranked second among 45 medicinal plants evaluated for their antioxidant capacity using ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays (Li, Wong, Cheng, & Chen, 2008). Since it is believed that lipid peroxidation is one of the causes of the occurrence of cardiovascular disease (Arts, Hollman, & Feskens, 2001) and cancer (Takabe et al., 2001), its high inhibition by extracts of our plants may repre-

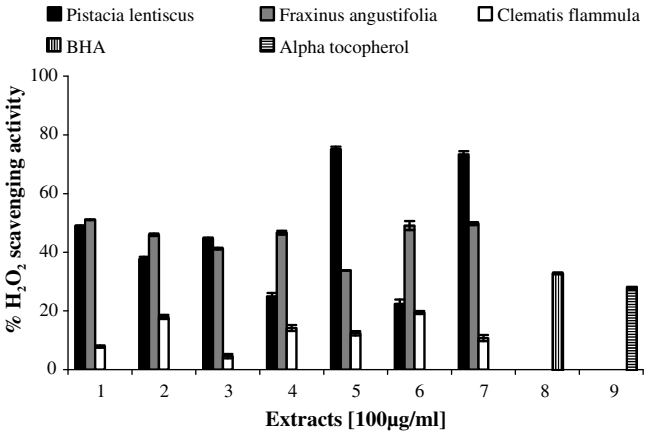

Fig. 3. H_2O_2 scavenging activity of extracts from *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* at 100 µg/ml (1. Ethanol, 2. Ethyl acetate, 3. Aqueous/ethyl acetate, 4. Hexane, 5. Aqueous/hexane, 6. Chloroform, 7. Aqueous/chloroform, 8. BHA and 9. Tocopherol).

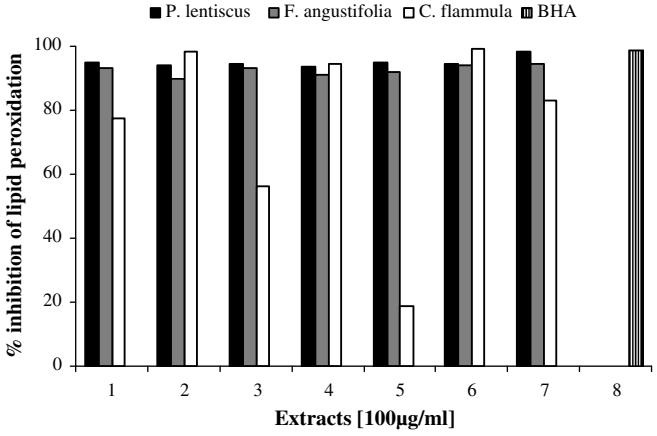

Fig. 4. % inhibition of linoleic acid peroxidation by extracts of *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula* at 100 µg/ml. (1. Ethanol, 2. Ethyl acetate, 3. Aqueous/ethyl acetate, 4. Hexane, 5. Aqueous/hexane, 6. Chloroform, 7. Aqueous/chloroform and 8. BHA).

sent an indicator of their high therapeutic potential. Furthermore, it has been shown that flavonoids have the capacity to terminate the chain reaction of lipid peroxidation by scavenging the peroxyl radical LOH. (Takahama, 1983). According to some studies, this activity is related to the number of hydroxyl groups (Cao, Sofic, & Prior, 1997) found mostly in aqueous extracts, consolidating our results regarding *Pistacia lentiscus* and *Fraxinus angustifolia*. However, the results concerning the inhibition of lipid peroxidation by organic extracts of *Clematis flammula* are in contradiction to the previous studies, suggesting that the mechanisms involved in lipid peroxidation, such as lipophilicity and membrane partitioning ability are more complex than a simple scavenging activity of peroxyl radical (Ollila, Halling, Vuorela, Vuorela, & Slotte, 2002).

3.5. Determination of total phenols, flavonoids and tannins

In an attempt to establish a potential relationship with different activities, we have determined the amount of phenolic compounds in various extracts tested. From the results summarised in Table 1, we can easily conclude that *Pistacia lentiscus* is rich in tannins and poor in flavonoids, whereas *Fraxinus angustifolia* and *Clematis flammula* are also poor in flavonoids, but just moderately rich in tannins.

Studies on antioxidant capacity and determination of phenols involving Algerian plants are extremely scarce. In fact, up to date, only two studies reported the antioxidant potential of Saharan plants. Mansouri, Embarek, Kokkalou, and Kefalas (2005) evaluated the phenolic profile and the antioxidant activity of seven varieties of the Algerian ripe date palm fruit (*Phoenix dactylifera*), using the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH.). They concluded that all the varieties tested exhibited high antioxidant activity despite their poorness in phenols, compared to other types of fruits. More recently, Djeridane and his colleagues (2006) investigated the antioxidant capacity of 11 medicinal Saharan plants used to treat gastric and inflammatory problems, using the stable radical 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\cdot+}$) and they concluded that most of the plants tested showed good antioxidant capacity which correlated positively with their phenol contents. DPPH scavenging activity and determination of polyphenolic content of seven Croatian *Stachys* taxa revealed that *S. recta*. sub. recta and *S. palustris* showed high scavenging activity (even higher than the reference, rutin) but poor correlation with total flavonoids (Vundac, Brantner, & Plazibat, 2007). On the other hand, a high correlation was found between antioxidant capacity and total phenols of methanol extracts from 45 medicinal plants (Li et al., 2008).

Having used several solvents, we found that the best yields of active compounds, especially tannins, were obtained in aqueous extracts. This is in agreement with a recent study on *Pistacia vera*, where it was found that the yield in total phenols depended on the method and the choice of solvent, and that the highest amount was obtained in water extracts (34.7 mg of tannic acid equivalent/g of plant powder) (Goli, Barzegar, & Sahari, 2005). Topçu and his colleagues (2007) found that the flavonoid contents of acetone and methanol extracts of *Pistacia terebinthus* are 5.49 and 22.60 μg quercetin equivalents/mg extract, respectively. While these results are not far from ours, discrepancies can be explained by the type of soil and microclimate.

Our results indicate the presence of a strong correlation between the reducing power and total phenols for *Pistacia lentiscus* ($r = 0.96$) and *Fraxinus angustifolia* ($r = 0.90$), respectively, which is in agreement with previous findings (Kaur & Kapoor, 2002; Odabasoglu et al., 2004). We also notice a strong correlation between the DPPH scavenging activity of extracts from *Fraxinus angustifolia* ($r = 0.97$) and *Clematis flammula* ($r = 0.85$) and their content in

Table 1
Determination of total phenols, flavonoids and tannins in extracts of *Pistacia lentiscus*, *Fraxinus angustifolia* and *Clematis flammula*

Extracts	Total phenols (mg Eq Catechin/g extract)			Flavanoids (mg Eq Quercetin/g extract)			Tannins (mg Eq Tannic Acid/g extract)		
	P. lentiscus Leaves	*F. angustifolia* Bark	*C. flammula* Leaves	*P. lentiscus* Leaves	*F. angustifolia* Bark	*C. flammula* Leaves	*P. lentiscus* Leaves	*F. angustifolia* Bark	*C. flammula* Leaves
Ethanol	136.25 ± 18.9	142.37 ± 7.48	19.65 ± 3.61	12.93 ± 1.69	6.6 ± 0.08	05.15 ± 0.10	909.4 ± 42.61	157.93 ± 34.48	86.33 ± 10.82
Ethyl acetate									
Organic fraction	75.01 ± 9.18	113.78 ± 8.27	55.08 ± 20.2	13.12 ± 0.12	6.2 ± 0.28	13.05 ± 0.79	632.1 ± 45.37	112.63 ± 9.54	260.47 ± 47.05
Aqueous fraction	40.65 ± 11.39	215.7 ± 14.16	14.70 ± 13.12	18.45 ± 1.2	4.93 ± 0.67	05.09 ± 0.05	852.2 ± 41.14	177.22 ± 16.91	1143.76 ± 01.30
Hexane									
Organic fraction	24.12 ± 8.3	15.53 ± 1.74	00.00 ± 2.38	10.06 ± 0.59	3.49 ± 0.05	08.52 ± 0.22	830.4 ± 62.19	379.07 ± 37.17	1280.52 ± 76.72
Aqueous fraction	452.95 ± 15.9	59.72 ± 3.4	39.14 ± 0.56	41.5 ± 0.91	3.49 ± 0.19	08.43 ± 0.06	773.9 ± 75.71	773.91 ± 75.71	65.10 ± 12.93
Chloroform									
Organic fraction	47.49 ± 16.88	100.17 ± 5.12	11.54 ± 03.33	7.74 ± 0.25	4.49 ± 0.14	19.81 ± 2.22	579.1 ± 34.84	142.18 ± 52.64	259.64 ± 23.59
Aqueous fraction	407.73 ± 1.53	242.71 ± 10.19	53.02 ± 03.30	44.25 ± 0.87	12.22 ± 0.3	12.05 ± 0.39	997.8 ± 39.08	187.69 ± 55.71	4.94 ± 03.80

Results are expressed as mean of 3 experiments ± SD.

total phenols. Concerning H_2O_2 scavenging activity, a strong correlation was established with the flavonoid content of *Pistacia lentiscus* ($r = 0.94$) and *Clematis flammula* ($r = 0.85$). These results strongly suggest that phenol compounds play an important role in the beneficial effects of medicinal plants and may be the basis of future substances that can be used in food storage and conservation.

4. Conclusions

Pistacia lentiscus, *Fraxinus angustifolia* and *Clematis flammula* are largely used in Algerian traditional medicine to treat a wide range of inflammations. In this study, we report, for the first time, their potential antioxidant capacity. The results indicate that *Pistacia lentiscus* was the most potent since it exhibited outstanding reducing power, scavenging activity against DPPH and H_2O_2 and inhibited strongly lipid peroxidation whereas *Fraxinus angustifolia* showed high DPPH scavenging activity and mild reducing power and H_2O_2 scavenging activity. *Clematis flammula* is ranked third among the three plants regarding reducing power, DPPH and H_2O_2 scavenging activities; nevertheless, it showed a significant inhibition of lipid peroxidation. Moreover, the same aqueous fractions from *Pistacia lentiscus* and *Fraxinus angustifolia* showed the highest activities, suggesting that active hydrogen-donating compounds are water soluble. In the case of *Pistacia lentiscus*, the best overall antioxidant capacity has been found in the aqueous fractions from hexane and chloroform extractions. On the other hand, for *Fraxinus angustifolia*, the highest antioxidant capacity has been assigned to the aqueous fraction from chloroform extraction. However, in the case of *Clematis flammula*, aqueous fractions from chloroform and hexane have shown the best reducing power and DPPH scavenging activities but it is the chloroform extract that has shown the best scavenging activity against H_2O_2 and inhibition of lipid peroxidation. Positive correlations were established between phenols and various activities, strongly suggesting that the observed activities of the three plants may be ascribed to their phenol compounds, which could be responsible, at least partly, for the obtained antioxidant activity. Moreover, the antioxidant activities of the plant extracts have overwhelmed, in many cases, that of BHA, a well known synthetic food additive. Therefore, the identification of specific phenolic compounds responsible for the high antioxidant activities which can be very beneficial for use as food additives represents one of our future aims.

Acknowledgements

This work was supported by a grant from the Ministry of High Education and Scientific Research of Algeria. We also wish to thank Said Bouadam and Farid Bekdouche (Associate Professors at the University of Bejaia) for plant identification.

References

Arts, I. C., Hollman, P. C., & Feskens, E. J. (2001). Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: The Zutphen Elderly Study. *American Journal of Clinical Nutrition, 74*, 227–232.

Baratto, M. C., Tattini, M., Galardi, C., Pinelli, P., Romani, A., Visiolid, F., et al (2003). Antioxidant activity of Galloyl quinic derivatives isolated from *Pistacia lentiscus* leaves. *Free Radical Research, 37*(4), 405–412.

Cao, G., Sofic, E., & Prior, R. L. (1997). Antioxidant and prooxidant behavior of flavonoids. Structure–activity relationships. *Free Radical Biology and Medicine, 22*(5), 749–760.

Cheesman, K., & Slater, H. (1993). An introduction to free radicals biochemistry. *British Medical Bulletin, 49*, 481–493.

Chiang, H., Lo, Y., & Lu, F. (1993). Xanthine oxidase inhibitors from the leaves of *Alsophila spinulosa* (Hook) Tryon. *Journal of Enzyme inhibition, 8*(1), 61–71.

Djeridane, A., Yousfi, M., Nadjemi, B., Boutassouna, D., Stocker, P., & Vidal, N. (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chemistry, 97*(4), 654–660.

Elliot, A. J., Scheiber, S. A., Thomas, C., & Pardini, R. S. (1992). Inhibition of glutathione reductase by flavonoids. *Biochemistry and Pharmacology, 44*, 1603–1608.

Ferrali, M., Signorinis, C., Caciotti, B., Sugherini, L., Ciccoli, L., Giachetti, D., et al (1997). Protection against oxidative damage of erythrocyte membranes by the flavonoid quercetin and its relation to ion chelating activity. *FEBS Letters, 416*, 123–129.

Finkel, T. (1998). Oxygen radicals and signaling. *Current Opinion in Cell Biology, 10*, 248–253.

Goli, A. H., Barzegar, M., & Sahari, M. A. (2005). Antioxidant activity and total phenolic compounds of pistachio (*Pistacia vera*) hull extracts. *Food Chemistry, 92*, 521–525.

Hagerman, A. E., & Butler, L. G. (1978). Protein precipitation method for the quantitative determination of tannins. *Journal of Agriculture and Food Chemistry, 26*, 809–812.

Halliwell, B., Gutteridge, J., & Cross, C. (1992). Free radicals, antioxidants, and human disease: How are we now? *Journal of Laboratory and Clinical Medicine, 119*(6), 598–620.

Han, S. S., Lo, S. C., Choi, Y. W., Kim, J. H., & Back, S. H. (2004). Antioxidant activity of crude extracts and pure compounds of *Acer ginnala* Max. *Bulletin of the Korean Chemical Society, 25*(3), 389–391.

Hancock, J., Desikan, R., & Neill, S. (2001). Role of reactive oxygen species in cell signaling pathways. *Biochemical and Biomedical Aspects of Oxidase Modification, 29*, 345–350.

Harborne, J. B., & Williams, C. A. (2000). Advances in flavonoid research since 1992. *Phytochemistry, 55*, 481–504.

Hirasa, K., & Takemasa, M. (1998). *Spice science and technology*. New York: Marcel Dekker.

Khan, M. A., & Baseer, A. (2000). Increased malondialdehyde levels in oronary heart disease. *Journal of Pakistani Medical Association, 50*, 261–264.

Kaur, C., & Kapoor, H. (2002). Antioxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology, 37*, 153–161.

Katalynic, V., Milos, M., Kulisic, T., & Jukic, M. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry, 94*, 550–557.

Kirby, A. J., & Schmidt, R. J. (1997). The antioxidant activity of Chinese herbs for eczema and of placebo herbs-I. *Journal of Ethnopharmacology, 56*, 103–108.

Knekt, P., Jârvinen, R., Reunanen, A., & Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: A cohort study. *British Medical Journal, 312*, 478–481.

Kumaran, A., & Karunakaran, R. J. (2007). Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. *Food Chemistry, 100*(1), 356–361.

Li, H. B., Wong, C. C., Cheng, K. W., & Chen, F. (2008). Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *LWT, 41*, 385–390.

Lowman, M. D., & Box, J. D. (1983). Variation in leaf toughness and phenolic content among five species of Australian rain forest trees. *Australian Journal of Ecology, 8*, 17–25.

Maksimovic, Z., Malencié, N. D., & Kovacevié, N. (2005). Polyphenol contents and antioxidant activity of Maydis stigma extracts. *Bioresource Technology, 96*(8), 873–877.

Mansouri, A., Embarek, G., Kokkalou, E., & Kefalas, P. (2005). Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*). *Food Chemistry, 89*(3), 411–420.

Masuda, T., Yonemori, S., Oyama, Y., Takeda, Y., Tanaka, T., Andoh, T., et al (1999). Evaluation of the antioxidant activity of environmental plants: Activity of the leaf extracts from seashore plants. *Journal of Agriculture and Food Chemistry, 47*(4), 1749–1754.

Mazur, A., Bayle, D., Lab, C., Rock, E., & Rayssiguier, Y. (1999). Inhibitory effect of procyanidin-rich extracts on LDL oxidation in vitro. *Atherosclerosis, 145*, 421–422.

Middleton, E., & Kandaswami, C. (1992). Effects of flavonoids on immune and inflammatory cell functions. *Biochemistry and Pharmacology, 43*, 1167–1179.

Middleton, P., Stewart, F., Al-Qahtani, S., Egan, P., O'Rourke, C., Abdulrahman, A., et al (2005). Antioxidant, antibacterial activities and general toxicity of *Alnus glutiosa*, *Fraxinus excelsior* and *Papaver rhoeas*. *Iranian Journal of Pharmaceutical Research, 2*, 81–86.

Mora, A., Paya, M., Rios, J. L., & Alcaraz, M. J. (1990). Structure–activity relationships of polymethoxyflavones and other flavonoids as inhibitors of non-enzymic lipid peroxidation. *Biochemistry and Pharmacology, 40*, 793–797.

Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., et al (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*(2), 145–171.

Nagao, A., Seki, M., & Kobayashi, H. (1999). Inhibition of xanthine oxidase by flavonoids. *Bioscience, Biotechnology and Biochemistry, 63*(10), 1787–1790.

Nakayama, T., Yamaden, M., Osawa, T., & Kawakishi, S. (1993). Suppression of active oxygen-induced cytotoxicity by flavonoids. *Biochemical Pharmacology, 45*, 265–267.

Nakayama, T. (1994). Suppression of hydroxyperoxide-induced cytotoxicity by polyphenols. *Cancer Research, 54*, 1991s–1993s.

Osawa, T., & Namiki, M. (1981). A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agricultural Biological Chemistry, 45*(3), 735–739.

Odabasoglu, F., Aslan, A., Cakir, A., Suleyman, H., Karagoz, Y., Halici, M., et al (2004). Comparison of antioxidant activity and phenolic content of three lichen species. *Phytotherapy Research, 18*, 938–941.

Ollila, F., Halling, K., Vuorela, P., Vuorela, H., & Slotte, J. P. (2002). Characterization of flavonoid-biomembrane interactions. *Archives of Biochemistry and Biophysics, 399*, 103–108.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition, 44*, 307–315.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogen, 10*, 1003–1008.

Sastre, J., Pallardo, F. V., & Vina, J. (2000). Mitochondrial oxidative stress plays a key role in aging and apoptosis. *IUBMB Life, 49*(5), 427–435.

Sultana, B., Anwar, F., & Przybylski, R. (2007). Antioxidant activity of phenolic components present in barks of *Azadirachta indica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam, trees. *Food Chemistry, 104*(3), 1106–1114.

Takabe, W., Niki, E., Uchida, K., Yamada, S., Satoh, K., & Noguchi, N. (2001). Oxidative stress promotes the development of transformation: Involvement of a potent mutagenic lipid peroxidation product, acrolein. *Carcinogenesis, 22*, 935–941.

Takahama, U. (1983). Redox reactions between kaempferol and illuminated chloroplasts. *Plant Physiology, 71*(3), 598–601.

Tepe, B., Sokmen, M., Akpulat, H. A., Yumrutas, O., & Sokmen, A. (2006). Screening of antioxidative properties of the methanolic extracts of *Pelargonium endlicherianum* Fenzl., *Verbascum wiedemannianum* Fisch. and Mey., *Sideritis libanotica* Labill. subsp. Linearis (Bentham) Borm., Centaurea mucronifera DC. and *Hieracium cappadocicum* Feyn from Turkish flora. *Food Chemistry, 98*, 9–13.

Topçu, G., Ay, M., Bilici, A., Sarikurkcu, C., Ozturk, M., & Ulubelen, A. (2007). A new flavone from antioxidant extracts of *Pistacia terebinthus*. *Food Chemistry, 103*, 816–822.

Vellosa, J. C. R., Khalil, N. M., Formenton, V. A. F., Ximenes, V. F., Fonseca, L. M., Furlan, M., et al (2006). Antioxidant activity of *Maytenus ilicifora* root bark. *Fitoterapia, 77*, 243–244.

Vundac, V. B., Brantner, A. H., & Plazibat, M. (2007). Content of polyphenolic constituents and antioxidant activity of some *Stachys* taxa. *Food Chemistry, 104*, 1277–1281.

Whysner, J., Wang, C. X., Zang, E., Iatropoulos, M. J., & Williams, G. M. (1994). Dose response of promotion of butylated hydroxyanisole in chemically initiated tumors of the rat fore stomach. *Food and Chemical Toxicology, 32*, 215–222.