International Food Research Journal 23(3): 948-953 (2016)

Journal homepage: *http://www.ifrj.upm.edu.my*

Effects of extraction solvents on phenolic content and antioxidant properties of *Pistacia atlantica* Desf fruits from Algeria

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Article history

Received: 16 June 2014
Received in revised form: 14 September 2015
Accepted: 28 September 2015

Keywords

Pistacia atlantica
Polyphenolic compounds
Antioxidant activity
Reducing power
Free radical scavenging assay

Abstract

The dry fruits of *Pistacia atlantica* were extracted with solvents of various polarity. Phenolic contents were determined using the Folin-Ciocaleu method, the reducing power (FRAP) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity were also determined. The total phenolic contents and the antioxidant activity are tightly dependent of the extracting solvent. The higher polyphenol content was observed for the crude methanolic extract, with value of 285.95 ± 10.25 mg gallic acid equivalent per gram of dry matter (GAE/ g DW). A high correlation was observed between phenolic content and antioxidant activity.

Introduction

Pistacia atlantica Desf. Subsp *atlantica* is a tree from Ancardiaceae family, which can reach over 15 m in height and grows in arid and semi-arid areas of Algeria, its vernacular name is "Butom". *P. atlantica* is valued because it is the source of mastic gum, exudates which strengthens gums, deodorizes breath, fights coughs, chills and stomach diseases (Bellakhder, 1997). Moreover, the galls of *P. atlantica* which are edible and sold in markets are used as an embalming gradient by rural habitants (Gourine *et al.*, 2010). The aerial parts and/or resin of plant has been also used in traditional medicine for the treatment of eczema, paralysis, diarrhea, throat infections, renal stones, jaundice, asthma, stomach-ache, and also as an astringent and a pectoral stimulant (Peksel *et al.*, 2013). This species is known for their potential antioxidant properties (Hatamnia *et al.*, 2014; Rezaie *et al.*, 2015), and also for their antidiabetic (Kasabri *et al.*, 2011), antimicrobial (Hosseini *et al.*, 2013), protoscolicidal (Mahmoudvand *et al.*, 2015), anti-inflammatory and cytotoxic activities (Sifi *et al.*, 2015, Minaiyan *et al.*, 2015).

In Algeria, the fruit, nammed Elkhodiri, have a high level in oil (39.80%) and in protein (10.39%). Locally, the oil, mixed with crushed date and whey, is eaten at any hour of the day (Benhassaini *et al.*, 2007). Chemicals studies on *P. atlantica* deals with flavonoids (Kawashty *et al.*, 2000; Pietta, 2000), fatty acids and triglycerides (Yousfi *et al.*, 2005; Benhassaini *et al.,* 2007; Farhoosh *et al.*, 2008;), chemical composition of the oleoresin (Delazar *et al.*, 2002; Delazar *et al.*, 2004; Benhassaini *et al.*, 2008), and chemical composition of the essential oils (Barrero *et al.*, 2005; Tzakou *et al.*, 2007; Mecherara-Idjeri *et al.*, 2008; Gourine *et al*, 2010). A new hispolone compound has been isolated from the methanolic extract (Yousfi *et al.*, 2009).

In a recent work by our research group (Belyagoubi-Benhammou *et al.*, 2014a, b), the antioxidant properties of fruits extracts of *P. atlantica* from Algeria were investigated. In this work, we continue to estimate the influence of extraction solvents on the phenolic profile and the antioxidant activity in fruits extracts obtained with solvents of various polarity. The temperature effect on the DPPH antiradical activity was also evaluated.

Materials and Methods

Chemical reagents

Methanol and 2,2-Diphenyl-1-picrylhydrazyl were purchased from Fluka Chemie (Buchs,

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Switzerland), Folin Ciocalteau was provided by Sigma-Aldrich Chemie (Germany), L-(+)-ascorbic acid was obtained from Merck (Darmstadt-Germany). All other chemicals and solvents were of analytic grade and obtained from Fluka.

Plant material

Fruits of *P. atlantica* Desf. were collected in Mai 2007 in the area of Ain Fezza, near Tlemcen, in the northern part of Algeria. The plant material identification was carried out at the laboratory of botany (University of Tlemcen), where a voucher specimen (No. 1784) has been deposited. The identification was done according to the New Flora of Algeria (Quezel and Santa, 1963). The fruits were dried in a shadowy place at room temperature, packed in paper bags and stored for future uses at the Laboratory of Natural Products (Department of Biology, Faculty of Sciences, University of Tlemcen, Algeria).

Extraction procedure

P. atlantica fruits were dry in an oven at 40°C for 24 h. The dry material was crushed into powder in a mortar and then macerated with solvents of increasing polarity: petroleum ether, chloroform, acetone, methanol and water. In a typical procedure, the dried power (1 g) was added to 20 ml of solvent, and gently stirred for 48 hours. After filtration through Whatman no1 filter paper, the solvent was eliminated under reduced pressure in a rotary evaporator at 60°C. The residue (crude extract) was dissolved in 3 mL of methanol for analysis. The same procedure was applied either for petroleum ether, or chloroform, or acetone or methanol, or water.

Total phenolic compounds

The Total Phenolic Content (TPC) was determined by spectroscopic method using the "Folin-Ciocalteu" assay (Singleton and Rossi, 1965). This method is generally considered to be the best suited for the determination of total phenolic compounds, including tannins. In a typical experiment, 200 µL of the final methanolic solution were mixed with 1 mL of Folin-Ciocalteu reagent, diluted 10 times, and a solution of sodium carbonate (0.8 mL, 7.5%). After gentle stirring for 30 min, the absorbance was determined at 765 nm and compared to a standard curve with gallic acid. The TPC was expressed as mg of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

Reducing power assay (Ferricyanide method) or FRAP

The ferric-reducing antioxidant power assay (ferricyanide method) or FRAP, is based on the formation of Prussian Blue. In the presence of antioxidants, Fe(III) is reduced into Fe(II) that forms the intensely colored Prussian Blue of which concentration can be determined from its absorbance at 700 nm. The reducing power of the extract was determined according to the Oyaizu method (1986) as follows: Various amount of extracts in distilled water (0.05; 0.1; 0.15; 0.2; 0.25 mg/mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide solution (2.5 mL, 1% $K_3[Fe(CN)_6]$). The mixture was incubated for 20 min at 50°C, and then, trichloroacetic acid (2.5 mL, 10%) was added. After centrifugation at 3000 rpm for 10 min, an aliquot volume of the supernatant phase (2.5 mL) was mixed with distilled water (2.5 mL) and a freshly prepared solution of $FeCl_3$ (0.5 mL, 0.1%). The absorbance was monitored at 700 nm and ascorbic acid was used as standard.

DPPH radical scavenging activity

The DPPH radical scavenging activity assay used in this paper is close to the method reported by Sanchez-Moreno *et al.* (1998). In a typical procedure, 50 µL of the extracts in methanol was mixed with 1950 µL of $DPPH^{\bullet}$ methanolic solution (0.025 g/L). The disappearance of the deep purple colour of the $DPPH^{\bullet}$ radical was monitored at 515 nm to reach finally a plateau value at the end of the reaction. The activities of the different extracts were compared to ascorbic acid as the reference antioxidant.

The time needed to reach the steady state depends of the chemical structure of the antioxidant. It is possible to define the Efficient Concentration or EC_{50} as the quantity of antioxidant needed to half the initial DPPH• concentration and may be expressed in mg dry extract/g $DPPH^{\bullet}$. For reason of clarity, it is also possible to define the Anti-Radical Power or $ARP = 1/EC_{50}$ that implies that the larger the ARP, the more efficient the antioxidant.

The time to reach the EC_{50} concentration is noted TEC_{50}. The Antiradical Efficiency AE may be defined as follows:

$$AE = 1/EC_{50} \times T_{EC50}$$

Temperature effect on the DPPH antiradical activity

The DPPH radical scavenging activity was determined after 30 min at four different temperatures in the water bath (25, 50, 75 and 100°C) (Rehma *et al.*, 2003).

Statistical analysis

Data were reported as means standard values from triplicate determinations. Correlation analyses of antioxidant activity were carried out using the correlation and regression programme in Origin 6 and Tcwin 2.

Results and Discussion

Extraction yields and total phenolic content (TPC)

In the present study, the extracts are generally viscous materials with a strong pleasant smelling odour. The yields of extracted material and the total phenolic contents are reported in Table 1. The yield of extracted material varies from 5.68% (chloroform) to 36.4% (crude methanolic extract). As expected from the literature, methanol is the most effective solvent for extraction of antioxidants from plant materials (Arabshahi-Delouee and Urooj, 2007). Not surprisingly, the TPC increases with the polarity of the extracting solvent: the highest value was observed for methanol (285.95 mg GAE/g DM) and the lowest one for petroleum ether (2.30 mg GAE/g DM).

Antioxidant activity

The FRAP IC_{50} from the different extracts was determined using a linear regression extrapolated to 0.5 absorbance unit (Table 2). The following sequence is observed: ascorbic acid > methanol > water > acetone > chloroform > petroleum ether. As expected, the petroleum ether extract had the lowest reducing power (IC_{50} = 6.91 ± 1.13 mg/mL) in this series.

The FRAP from the different extracts are positively correlated with the TPC in the extracts, a correlation that has been previously reported by several authors (Arabshahi-Delouee and Urooj, 2007). The mode of action of polyphenolic antioxidant molecules is to terminate radical chain reactions by converting the radicals into more stable products, either by monoelectronic transfer or by radical H or alkyl/aryl groups transfer (Dorman *et al*., 2003). The reducing power of the extract is an indicator of its antioxidant properties (Meir *et al*., 1995).

The antioxidant activities of the extracts should be determined using complementary methods, because of the variety of chemical structures of molecules extracted and because of the diversity of their mechanisms of action that cause the macroscopic so-called "antioxidant activity" of the extracts.

The $DPPH^{\bullet}$ radical scavenging activities are reported in Table 2. The higher the observed EC_{50}, the lower is the DPPH radical scavenging activities. Values from 42.05 to 4147.6 mg/g DPPH, were observed for acetone extract and petroleum ether extract, respectively. They may be compared to value of 39.53 mg/g $DPPH^{\bullet}$ for ascorbic acid. The weak DPPH radical scavenging activity of the petroleum ether extracts may be explained by the fact that many antioxidants have polar phenolic groups, and may also be present as more polar glycosidic derivatives. Therefore, it is not surprising that non-polar solvents such as petroleum ether are not able to extract them. The DPPH radical scavenging activities are as follows: ascorbic acid > acetone extract > water extract > methanol extract > chloroform extract > petroleum ether extract. It can be concluded that the higher the polarity of the solvent, the higher the DPPH radical scavenging activity of the extract. Such observations were previously reported in the literature (Turkmen *et al*., 2006).

Table 1. The yields and total phenolic contents of *P. atlantica* fruits extracts

Sample extracts	Yield (%)	Phenolic content (mg GAE/g DM)
Petroleum ether	6.22	2.30 ± 0.10
Chloroform	5.68	10.23 ± 0.44
Acetone	14.65	31.86 ± 0.63
Methanol	36.40	285.95 ± 10.25
Water	27.10	129.16 ± 0.65

Values expressed are means ± SD of three parallel measurements. GAE, gallic acid equivalents.

The polar fractions may have polyhydroxylated phenolic compounds able to present cooperative effects and/or synergistic effect with other compounds in connection with the fact that their antioxidant properties is dependent on the arrangement and position of functional groups on the ring structure (Yu *et al*., 2005; Cai *et al*., 2006). According to the solvents used for extraction, the antiradical efficiency (AE) of the extracts is in the following decreasing order: ascorbic acid > water > methanol > acetone > chloroform > petroleum ether (Table 2).

The AE of *P. atlantica* fruits from methanolic extract (0.10×10^{-3}) is least comparable to that of *P. atlantica* leaves (0.138×10^{-3}) but weaker as compared to that of *P. lentiscus* (0.328×10^{-3}) (Benhammou *et al*., 2007). This result may be attributed to the presence of phenolic and flavonoids compounds in *Pistacia* species such as quercetin, α-tocopherols, gallic acid and its derivatives (Topçu *et al*., 2007).

The AE of gallic acid (2.62×10^{-3}), tannic acid (0.57×10^{-3}), caffeic acid (2.75×10^{-3}), ascorbic acid (11.44×10^{-3}), quercetin (0.19×10^{-3}), BHA (0.10×10^{-3}) and α-tocopherols (0.52×10^{-3}) were reported by Sanchez-Moreno *et al.* (1998). Gallic acid showed the highest free radical scavenging capacity. A molecule of gallic

Table 2. Determination the IC_{50}, EC_{50} concentrations values and the Antiradical Efficiency in various solvent extracts

Sample extracts	IC_{50} (mg/mL)	EC_{50} (mg Antioxidant/ g DPPH)[a]	R^2	Time (T_{EC50}) (min)[b]	R^2	AE
Petroleum ether	6.91 ± 1.13	4147.6	0.938	150.20	0.943	0.01[c]
Chloroform	0.91 ± 0.05	597.42	0.994	47.24	0.998	0.35[c]
Acetone	0.43 ± 0.01	42.05	0.998	496.93	0.995	0.05[d]
Methanol	0.13 ± 0.00	47.4	0.998	255.94	0.999	0.10[d]
Water	0.16 ± 0.00	43.53	0.999	191.20	0.998	0.12[d]
Ascorbic acid	0.06 ± 0.00	39.53	0.917	0.61	0.893	41.74[d]

IC_{50} (mg/ mL) is an effective concentration at which the absorbance is 0.5.
[a] EC_{50} values were calculated from the residual percentages of DPPH plotted versus the extract concentrations (mg Antioxidant/ g DPPH).
[b] T_{EC50} were obtained by times at steady state versus the concentrations (mg Antioxidant/ g DPPH).
[c] Antiradical efficiency ($\times 10^{-4}$); d Antiradical efficiency ($\times 10^{-3}$).

Table 3. Effect the temperature on the EC_{50} concentrations and antiradical efficiency

T (°C)	EC_{50} (mg Antioxidant/g DPPH)	T_{EC50} (min)	Antiradical efficiency ($\times 10^{-3}$)
25	32.85	451.76	0.06
50	41.55	85.32	0.28
75	43.14	68.43	0.33
100	48.28	118.85	0.17

acid with its three-hydroxyl groups on the aromatic ring is able to reduce more than six molecules of DPPH radical (Brand-Williams *et al.*, 1995).

No linear correlation between reducing power method and total phenolic content ($R^2 < 0.95$). The correlation between DPPH radical scavenging activity (EC_{50}) and total phenolic content was not linear ($R^2 <$ 0.95) either. A similar result was previously observed by Karagozler *et al.* (2008). In opposite, Figure 1 demonstrate a positive and highly significant linear correlation ($R^2 = 0.997$) between IC_{50} values of reducing power and the EC_{50} of DPPH radical scavenging. It may indicate that the polyphenolic compounds of all tested extracts showed the same bioactive molecules and molecular mechanism for radical scavenging and reducing power.

Only crude methanolic extracts were used for the assessment of temperature effect on DPPH assay. The effect of the temperature is a very essential parameter in studies related to food industry, because it intervenes in the chain of several manufacturing processes, extraction and conservation of the foodstuffs. The search for optimized extraction methods of phenolic compounds and the environmental factors in particular temperature on the antioxidant activity is necessary to evaluate. As shown in Table 3, the EC_{50} concentrations increased proportionally with temperature increase, from 32.85 at 25°C to 48.28 at 100°C. As expected from a kinetic point of view, TEC_{50} decreased as the temperature increased. In

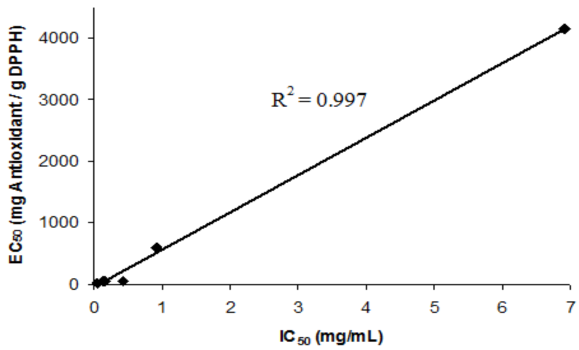

Figure 1. Positive correlation between the IC_{50} values of reducing power and EC_{50} of DPPH radical scavenging

addition, the introduction of parameter AE gives a clear appreciation concerning the evaluation of the antioxidant activity of DPPH scavenging. We note that the highest antioxidant activity expressed in AE increases according to temperature. This was deferred by literature, where the increase in DPPH scavenging activity was found during heat treatment (Li *et al.*, 2007). This phenomenon can be explained by an appearance of new aglycone molecules endowed with a strong antioxidant activity formed after hydrolysis under the heat treatment at less to 100°C for 30 min. Therefore, the higher free radical scavenging capacity of methanolic extract might be correlated to a heating–induced increase in the content and nature of these compounds. It is noted that the antioxidant activity of phenolics not mainly depends on the structure of aromatic ring, but is also affected by other factors such as glycosylation of aglycones and other H-donating groups (-NH, -SH) (Cai *et al.*, 2004; Cai *et al*., 2006).

Conclusion

In this study, the results indicate that the total polyphenol contents and antioxidant activity are highly dependent on the nature of solvent. Methanol and water extracts were found to have higher phenolic content and the best reducing power and DPPH scavenging activities. Therefore, the identification of specific phenolic compounds responsible for the high antioxidant activities, which can be very beneficial for use as food additives to preserve the lipids oxidation and to maintain the good quality of foodstuffs should be investigated.

Acknowledgements

We are gratefully to Pr. Ahcène BOUMENDJEL, Laboratory of Pharmacognosy, Equip "Synthesis, biological activity and relation of structure-activity of analogues of natural products", UMR-CNRS 5063, UFR of Pharmacy of Grenoble, University Joseph Fourier, France, for his assistance and useful recommendations, for critical reading and correction of the manuscript.

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