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Chemical Composition and Antioxidant Activity of Essential Oil of Leaves of *Pistacia atlantica Desf.* from Algeria

NADHIR GOURINE*, MOHAMED YOUSFI, BOUBAKEUR NADJEMI† and ISABELLE BOMBARDA‡
Laboratoire des Sciences Fondamentales à l'Université Amar TÉLIDJI de Laghouat, 03000 Laghouat, Algeria
Tel/Fax: (213)(29)900066; E-mail: n.gourine@mail.lagh-univ.dz

The essential oil obtained by hydrodistillation of leaves of *Pistacia atlantica* at a yield of 0.13 % (v/w) were analyzed by GC/MS. Thirty one compound was identified representing 89.46 % of the total essential oil. The main components were β-pinene (19.08 %), α-terpineol (12.82 %), bicyclogermacrene (8.15 %) and spathulenol (9.45 %). The antioxidant activity of the oil, was evaluated *in vitro* using two different assays: DPPH• (2,2-di-phenyl-1-picrilpydrazyl) free radical-scavenging and FRAP (ferric reducing antioxidant power). The result of the DPPH assay gives an IC_{50} value of 9.61 ± 0.7 mg/mL. The FRAP antioxidant activity test of the oil was expressed using two standards reference antioxidants (ascorbic acid and butylated-hydroxyanisole). The antioxidant expressed as butylated-hydroxyanisol equivalent antioxidant capacity (BEAC) has a value of 8.73 ± 0.3 mM which means that the oil is much more active than butylated-hydroxyanisole. While the ascorbic-acid equivalent antioxidant capacity (AEAC), has a value of 54.77 ± 1.14 µM.

Key Words: *Pistacia atlantica*, Essential oil, Antioxidant activity, Composition, Leaves.

INTRODUCTION

Plant-derived natural chemicals, known as secondary metabolites, are effective in their roles of protection, adaptation and pollination. Secondary metabolites are mainly used in food, pharmaceutical, chemical, cosmetic industries and agriculture[1,2]. Plant-derived natural products are abundant in nature. Many of them exhibit numerous biological activities and some can be employed as food additives. Synthetic antioxidants have been used in the food industry since the 1940s, but trends in many health-related industries tend to shift preferences to natural sources. Therefore, investigation of natural antioxidants has been a major research interest for the past two decades as many research groups and institutions have been screening plant materials for possible antioxidant properties[2].

†Laboratoire de biomasse, École Normale Supérieure, Kouba, Alger, Algeria.
‡Laboratoire de recherche des systèmes chimique complexes. Faculté des sciences et technique de Saint-Jérôme, Marseille, France.

The most widely synthetic antioxidants [butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl galate (PG) and tertiary butyl hydroquinone (TBHQ)] used in food have been suspected to cause or promote negative health effects[3,4]. For this reason, there is a growing interest in studies of natural additives as potential antioxidants.

The essential oils and extracts of many plant species have become popular in recent years and attempts to characterize their antioxidant activity and their use in pharmaceutical or foods processing are advised[5-7]. The essential oils have been of great interest for their potential uses as alternative remedies for the treatment of many infectious diseases, pharmaceutical alternative medicine and natural therapies[8,9].

The genus *Pistacia* (Anacardiaceae) is widely distributed in the Mediterranean area[10]. *Pistacia atlantica Desf.*, is a tree located in north Africa, which can reach over 15 m in height and grows in arid and semi-arid areas[11]. *Pistacia atlantica* is valued because it is the source of mastic gum, exudates which strengthens gums, deodorizes breath, fights coughs, chills and stomach diseases[12]. Moreover, the galls of *Pistacia atlantica* are used as an embalming gradient by rural habitants. They are also known in Arabic as "Butom" and are edible and sold in markets.

Due to lack of knowledge about antioxidant activity of *Pistacia atlantica* essential oil, this study was carried out in order to evaluate and assess the capacity of the essential oil of the leaves as bioactive antioxidants by employing two different and complementary assays *i.e.*, the DPPH free radical scavenging assay and the FRAP assay. The FRAP method measure only the hydrophilic antioxidants, while the DPPH method detect only those soluble in organic solvents, especially alcohols. Both chosen assays involve electronic transfer, but uses different chromogenic redox reagents with different standard potentials.

EXPERIMENTAL

Ethanol, sodium acetate trihydrate, 2,4,6-tripyridyl-s-triazine (TPTZ), hydrochloric acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Germany). Ascorbic acid and butylated hydroxyanisole (BHA) were obtained from Fluka (Switzerland). Acetic acid and sodium sulphate were obtained from Prolabo Merk. $Fe_2(SO_4)_3 \cdot 7H_2O$ was obtained from Panreac (Spain).

Fresh leaves, from the plant of *Pistacia atlantica Desf.*, were collected at the end of October 2006 from a wild area located 8 km outside the town of Laghouat 400 km south of the Algiers, in a place which belong to a Saharian Atlas, in a Mediterranean climate.

Preparation of samples: Extract of air-dried leaves in shadow at room temperature of *Pistacia atlantica* was prepared by water distillation using Clevenger apparatus for almost 7 h which yielded 0.13 % v/w. The obtained oil was dried over anhydrous sodium sulphate, filtrated and stored at 4 ºC until tested and analyzed.

GC/MS Analysis: The chemical composition of the *Pistacia atlantica* leaves essential oil (PA essential oil) was analyzed using a AGILENT 6890 GC/CMSD 5973 equipped with a capillary column HP5MS (30 m × 0.25 mm, 0.25 µm film

thickness) and a 70 eV EI Quadruapole detector. Helium was the carrier gas, at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 250 and 220 °C, respectively. Column temperature was initially at 60 °C held for 2 min, then gradually increased to 125 °C at a 2 °C/min rate, held for 2 min and finally increased to 220 °C at 5 °C/min held for 2 min. Diluted samples (1:100 v/v, in ethanol) of 1.0 µL were injected manually using splitless mode. Compound identification was verified based on relative retention time and mass spectra with those of the Computer library data of the GC-MS system (AGILENT).

DPPH Assay: The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant. DPPH solutions show a strong absorption band at 517 nm with a deep violet colour. The absorption vanishes and the resulting decolouration is stochiometric with respect to degree of reduction. The remaining DPPH, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant[13-17]. 50 µL of the extracted oil dilutions in ethanol was added to 1 mL of 500 µM solution of DPPH. After 0.5 h of incubation at room temperature, the absorbance was read against a blank at 517 nm (Shimadzu UV/Vis 1601 apparatus). Inhibition of DPPH free radical in percent (I %) was calculated as follows:

$$I\% = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \times 100\%$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

Extract concentration providing 50 % inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. All tests were carried out in triplicate. The α-tocopherol was used as a positive control.

FRAP Assay: Determination of ferric reducing antioxidant power FRAP is a simple direct test for measuring antioxidant capacity. This method was initially developed to assay plasma antioxidant capacity, but can be used with plant extracts too. The FRAP assay was performed as described by Benzie, Iris and Nilsson[18-20]. The FRAP reagent was prepared by mixing 300 mM of acetate buffer, pH 3.6 [3.1 g sodium acetate trihydrate, plus 16 mL glacial acetic acid made up to 1 L with distilled water]; 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 20 mM $Fe_2(SO_4)_3 \cdot 7H_2O$ in the ratio of 10:1:1 to give the working FRAP reagent. The working reagent was freshly prepared as required. For each test component, (100 µL) of several prepared dilutions of oil extract in ethanol 95 %, was added to a diluted FRAP reagent in methanol (1 mL FRAP mixed with 2 mL methanol) and the absorbance was read at 593 nm (Shimadzu UV/Vis 1601 apparatus), the absorption reading was performed at room temperature for a time period of 6 min, against a blank (FRAP diluted reagent previously prepared without the oil extract). Data were expressed relative to values obtained with BHA and ascorbic acid from calibration curves and then expressed as BHA and as ascorbic acid equivalents. All the tests were carried out in triplicate.

Statistical analysis: The results are reported as mean ± SD of three independent replicates. Statistical analysis of data was carried out by computer using both MS-Excel and Origin 5.0. Multiple comparison tests were used to analyze data. p Values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Extract of air-dried leaves of *Pistacia atlantica* prepared by water distillation (Clevenger apparatus) yielded: (0.13) % (v/w) and were obtained as brown colour oil with aromatic-spicy odour. The composition of *Pistacia atlantica* essential oil was analyzed by GC/MS. Thirty one components representing 89.46 % of the total oil were identified. The oil was found to be rich in monoterpene (45.57 %). Monoterpene alcohols represent (15.47 %). The main monoterpenes identified were β-pinene (19.08 %), α-terpineol (12.82 %), limonene (3.8 %) and β-myrcene (2.6 %). The total amount of sesquiterpenes in the essential oil were (30.34 %) with spathulenol (9.45 %), bicyclogermacrene (8.15 %), viridoflorol (5.29 %) and aromadendrene (2.3 %) as major constituents. The total percentage composition of sesquiterpene alcohols were estimated *ca*. 16.83 % (Table-1).

Leaves of many species of *Pistacia* have been investigated for the essential oil composition (*P. lentiscus, P. khinjuk, P. chinensis, P. terebinthus, P. palaestina, P. atlantica*). Comparison of previous studies shows a remarkable variability that seems to depend on plants species and plant organ. In the essential oil obtained from the leaves, some authors found a dominance of monoterpene hydrocarbons[21-26]. In particular, the main constituents of the most studied species, *P. lentiscus*, were α-pinene (Greece and France)[25-27], myrcene (Spain and Sicily)[21,25], δ-carene (Egypt)[23] and terpinen-4-ol, α-pinene, limonene and myrcene (Corsica)[26]. According to their intraspecific variability, the essential oil of *P. lentiscus* can be classified into three groups on the basis of their content of terpinen-4-ol, α-pinene, limonene and myrcene. In the essential oil of the galls of *P. integerrima*, monoterpenes dominated, with α-pinene, sabinene, β-pinene and limonene as the main components. Although *Pistacia palaestina* is a variety of *Pistacia terebinthus*, the composition of the essential oil of the leaves was very different[28]. In *Pistacia terebinthus*, the monoterpenes terpinen-4-ol, γ-terpinene, limonene and α-terpinene have been detected as the main compounds, while in *Pistacia palaestina* the main compounds were α-pinene and myrcene[29]. In Table-2, the main constituents of some *Pistacia* species are summarized to show the relationship between the compositions of the leaves in the same species. It is concluded that the main compositions of each oil of *Pistacia* species is different from the others ones. Further more if we compare the same part of the same species for example leaves of *P. lentiscus* from Turkey, Spain and Algeria, a different composition is found, which could be due to the difference of the climate and the region of the growth and may also be due to the tree itself. These differences in the compositions are generally responsible of the several medicinal uses, which are different country to country.

TABLE-1
CHEMICAL COMPOSITION OF P.A. LEAVES ESSENTIAL OIL

Peak no.	Compounds[a]	Retention time (min)	Composition[b] (%)
1	β-Pinene	3.55	19.08
2	Pyrrole, 1-methyl	3.78	2.04
3	β-myrcene	4.30	2.60
4	α-Terpinene	4.62	0.54
5	Limonene	4.99	3.80
6	β-Phellandrene	5.14	0.59
7	(*trans*) 2-Hexanal	5.26	1.03
8	γ-Terpinene	5.89	1.00
9	*p*-Cymene	6.33	0.54
10	Terpinolene	6.67	1.45
11	6-Methyl-5-hepten-one	7.81	0.44
12	3-Hexen-1-ol	8.95	1.33
13	2-Hexen-1-ol	9.48	0.33
14	Linalool	12.87	0.64
15	Bornyl acetate	13.57	3.35
16	Camphene	13.95	0.50
17	Terpineol-4	14.12	2.01
18	Aromadendrene	14.42	2.30
19	Gurjunene	14.85	0.70
20	Allo-Aromadendrene	15.25	0.90
21	α-Terpineol	16.31	12.82
22	Bicyclogermacrene	17.18	8.15
23	Germacrene B	19.15	1.46
24	Palustrol	21.23	0.93
25	Phenol	22.48	0.42
26	Ledol	23.21	0.42
27	Globulol	24.12	5.29
28	Viridiflorol	24.26	3.46
29	Spathulenol	25.10	9.45
30	Isospathulenol	26.85	1.67
31	Phytol	33.57	0.22
Total		–	89.46

[a]Compounds listed in order of elution from HP5 MS column.
[b]Relative area percentage (peak area relative to total peak area %).

On the basis of the present results and in comparison with other previous works carried out for the same tree, the composition percentage of the major components of essential oil of *Pistacia atlantica*, that grows in Algeria are quite different from those which grows in other countries, since the percentage of the major components of leaves oil of *P. atlantica* from Algeria were β-pinene (19.08 %), α-terpineol (12.82 %), bicyclogermacrene (8.15 %) and spathulenol (9.45 %) are different from those found in *P. atlantica* from Morocco:terpinen-4-ol (21.7 %) and elemol (20.0 %) and *P. atlantica* from Greece (Table-2).

TABLE-2
MAIN COMPOSITIONS OF LEAVES ESSENTIAL OIL OF SOME *Pistacia* SPECIES

Plant name	Country of origin	Main constituents of essential oil leaves extract	Reference
P. atlantica	Morocco	Terpinen-4-ol (21.7 %) Elemol (20.0 %)	30
P. atlantica	Greece "female tree"	Myrcene (17.8-24.8 %) Terpinen-4-ol (11.6-6.0 %)	31
P. atlantica	Greece "male tree"	Terpinen-4-ol (17.3 %) *p*-Menta-1(7),8-diene (41.1 %)	31
P. terebinthus	Turkey	Terpinen-4-ol (33.7 %) γ-Terpinene (9.3 %) α-Terpineol (8.1 %)	28
P. lentiscus	Turkey	Terpenin-4-ol (23.9 %) α-Terpineol (10.6 %) Limonene (10.6 %)	28
P. lentiscus	Spain	α-Pinene (11.0 %) β-Myrcene (19.0 %)	21
P. lentiscus	Algeria	Terpinen-4-ol (17.3-34.7 %) α-terpineol (10.4-11.0 %) Germacrene D (8.4-15.8 %)	32
P. lentiscus	Egypt	Car-3-ene (65.3 %)	23
P. khinjuk Stocks	Egypt	α-Pinene (18.4 %) Sabinene (13.5 %) Terpinen-4-ol (12.1 %)	23
P. chinensis Bunge	Egypt	(*trans*) β-Ocimene (38.8 %) Limonene (26.5 %)	23
P. palaestina Boiss.	Jordan	α-Pinene (63.1 %) Myrcene (13.3 %)	29

The free radical-scavenging activity was determined by the DPPH test. This test aims to measure the capacity of the extracts to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) formed in solution by donation of hydrogen atom or an electron[16]. If the oil extract has the capacity to scavenge the DPPH free radical the initial blue/purple solution will change to a yellow colour due to the formation of diphenylpicrylhydrazine. This reaction is used as a measure of the ability of the oil extract or any other antioxidant, such as α-tocopherol or ascorbic acid, to scavenge any free radical. Free radical scavenging activity of the oil extracts is concentration dependent and lower IC_{50} value reflects better protective action. A direct linear relationship was found between the concentration of the essential oil in the DPPH$^{\bullet}$ solution and the inhibition percentage with a correlation factor of R^2 = 0.9972 (Fig. 1). The value of each absorbance taken was the mean of three independent experiments. Using this linear equation, essential oil concentration providing 50 % inhibition was calculated and found equal to IC_{50} = (9.61 ± 0.7) mg/mL. For

α-tocopherol, which is used as a positive control, the value of IC_{50} was found (0.026 ± 0.006) mg/mL. Theses results indicate that the essential oil have a good radical scavenging antioxidant capacity in organic environment in term of DPPH assay.

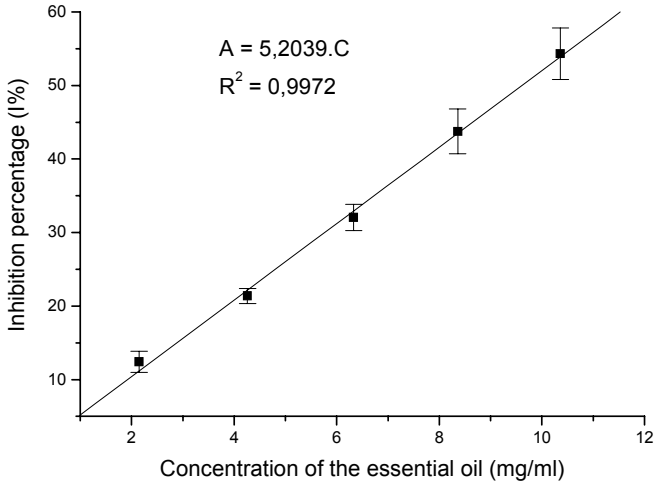

Fig. 1. Relation between the inhibition percentage and the concentration of the essential oil in the DPPH solution

The ferric reducing ability of plasma (FRAP) assay was used for assessing 'antioxidant power' of *Pistacia atlantica* essential oil. The antioxidant power of the leaves oil was compared with butylated-hydroxyanisol and with ascorbic acid as reference antioxidants. In this method the ferric reducing ability of antioxidant compound is measured. At low pH, ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex is reduced to the ferrous (Fe^{2+}) blue colour complex with an absorption maximum at 593 nm. Test conditions favour reduction of the complex and, thereby, colour development, provided that an antioxidant is present. The antioxidant activity expressed as butylated-hydroxyanisol equivalent antioxidant capacity value of (8.73 ± 0.3) mM. This value means that the essential oil has a very height antioxidant capacity (approximately over 8 times the antioxidant capacity of BHA). While the ascorbic acid equivalent antioxidant capacity has a value of (54.77 ± 1.14) µM. This value confirms the high antioxidant capacity of vitamin C in water solution. Finally, the present results indicate that the essential oil has a good antioxidant capacity, can be used in both water and organic solution environments.

Conclusion

The increased availability of food products, which has altered the nature of their production, quality and organoleptic characteristics, increased requirements for the quality and condition of the individual products and stimulated a demand in

consumer markets, is focusing on a high market value. The phytochemical investigation and evaluation of the antioxidant activities of leaves of *Pistacia atlantica* from Laghouat, Algeria, indicated important antioxidant activity of the extracted essential oil. This activity was estimated by employing two different and complementary assays *i.e.*, the DPPH free radical scavenging assay and the FRAP assay. The FRAP method measure only the hydrophilic antioxidants, while the DPPH method detect only those soluble in organic solvents, especially alcohols. Both chosen assays involve electronic transfer, but uses different chromogenic redox reagents with different standard potentials. The use of two assays has the advantage to follow the action of antioxidant activity of the essential oil compounds in two different solution environments (lipophile and hydrophile). The interpretation of the present results reveal that the value of IC_{50} evaluated for the DPPH assay indicate a weak antioxidant capacity in term of bleaching the free radicals present in organic solutions. The essential oil has a high antioxidant capacity in term of FRAP assay, which means that the oil can reduce better than BHA and the metal ions present in water solutions who are responsible for the oxidation of food components. Finally, it can be concluded that the essential oil of the leaves of *Pistacia atlantica* has both actions in reducing the effect of free radicals presents in organic solutions and in reducing the ion metals responsible of oxidation of foodstuffs which can be found in water solution environments.

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