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Pistacia Atlantica Desf., a Source of Healthy Vegetable Oil

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Abstract: *Pistacia atlantica*, which belongs to the Anacardiaceae family, is an important species for rural people in arid and semi-arid areas. The fruit, rich in oil, is used in traditional medicine for the treatment of various diseases. The oil extracted from this species growing in a northern area of Algeria and its fatty acid composition were previously studied. However, the largest areas where this species is present (traditional cultivation) is located in southern Algeria. Moreover, studies on oil fatty acid composition and essential oil were always conducted separately. This study was performed in order to assess the fatty acid and volatile organic compound composition of *P. atlantica* vegetable oil. The seeds were collected randomly from Djelfa (300 km South of Algiers, Algeria). Oil content and fatty acid composition were determined by Soxhlet extraction. The seeds contained high concentrations of oil (32–67%). The major fatty acids were oleic (39–49%), linoleic (23.6–31%), and palmitic (21.3–26.6%) acids. The ratio of polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) indicated that the content of unsaturated fatty acids was approximately three times higher than that of SFA. This ratio is widely used in epidemiological studies and research on cardiovascular diseases, diabetes, and metabolic syndrome. The ratios of ω-acids, i.e., ω-9/ω-6 and ω-6/ω-3, were 1.3–2 and 18.5–38.3, respectively. Crushed seeds were analyzed by headspace solid-phase microextraction (SPME) coupled with gas chromatography–mass spectrometry. More than 40 compounds were identified, mainly monoterpenes ($C_{10}H_{16}$), such as α-terpinene and terpinolene, but also sesquiterpenes ($C_{15}H_{24}$) at lower levels. The value of this species as a source of healthy oil rich in ω-3 acid and its effects on cardiovascular disease risk are discussed.

Keywords: *Pistacia atlantica*; oil content; fatty acid composition; Solid-Phase Micro-Extraction (SPME); volatile organic compounds

1. Introduction

Pistachio of Atlas (*Pistacia atlantica* Desf.) is a fast-growing species of the Anacardiaceae family. Its height may reach 20 meters. This species is cultivated in different soil types and tolerates abiotic stresses such as drought [1,2]. *P. atlantica* covers a large area spanning across North Africa, Middle East, Iran, and Afghanistan [3]. The fruits are edible drupes rich in oil, used in folk medicine for the treatment of various diseases and widely consumed as a nutrient by the local populations. The oil can be used in food, cosmetics, and for medicinal purposes [4,5]. The resin is used in the food industry to prepare chewing gum and is widely exploited in dentistry [6]. The resin and fruits are also used

traditionally for stomach aches, dyspepsia, peptic ulcer, and throat infections [7]. Other applications have been reported as insect repellent [8,9], expectorant, against asthma and chest diseases [10], plant fungi diseases [11], or in the treatment of non-tuberculous mycobacterial infections [12]. The essential oil from *Pistacia* fruit showed fumigant activity against *Callosobruchus maculatus*, protective effects against ethanol-induced gastric ulcer, and antibacterial activity against *Helicobacter pylori* [13,14].

In recent decades, advances have been made in research on the consumption of natural food to improve human health. The development of functional foods, nutraceuticals, branded foods, therapeutic foods, superfoods, and medicinal foods is influencing the current dietary habits [15]. Moreover, because of the growing demand of edible oil to satisfy the population needs and the important effects of oils on diseases prevention, oil production has witnessed a significant increase [16]. The health protective effects of oils are influenced by their fatty acid composition. Polyunsaturated fatty acids (PUFA) are necessary for the functioning of the brain and the eyes. They were shown to be effective in lowering total cholesterol levels, reducing the risk of breast cancer, and preventing cardiovascular and neurodegenerative diseases [17–21]. Western diets are in constant evolution, and the demand for new sources of oils containing polyunsaturated fatty acids has become a health issue [16,22,23].

Essential oils contain active compounds that can be used to obtain functional materials with antioxidant, antimicrobial, or pesticidal activities [24–26]. Recent studies have reported that essential oils could provide an interesting solution for pest management and bacterial and fungal growth control in food and agricultural products in order to prevent health risks. The phytochemical composition and traditional uses of Pistachio of Atlas were well documented by Mahjoub et al. [27]. Studies have investigated oil and fatty acid composition of pistachio seeds [28,29] as well as essential oil content and profile in pistachio leaves [8,30,31]. These studies focused on wild pistachio from northwest Algeria. There is no information about the composition of oil from the seeds cultivated in south Algeria, which is the most important zone of traditional cultivation of this species. Moreover, no reports are available on the volatile organic compounds (VOC) of the oil extracted from pistachio seeds. Even if this species remains underutilized, this information is important to enhance *Pistacia* oil uses. Solid-phase microextraction (SPME) can extract and pre-concentrate VOC from different matrices [28,32]. This method can be used for the characterization of the VOC from *P. atlantica* oil. Therefore, the aim of this study was to extract oil from pistachio harvested in Algeria and to characterize its composition in fatty acids and in volatile organic compounds.

2. Materials and Methods

2.1. Area of Seed Harvest and Plant Materials

Fruits of *P. atlantica* Desf. were collected, at full ripening, in Djelfa region (34°02′11″ North, 03°40′22″ East), 300 km South of Algiers (Algeria) from the beginning of October to November 2016. This region is characterized by an arid climate, with 185 mm of precipitations recorded in 2016 and an average temperature of 15.4 °C. The detailed climatic conditions are presented in Table 1.

The fruit harvest area extends over three hectares. The fruits were harvested randomly, according to the method of transect, from 30 trees and were kept in the laboratory at 4 °C until extraction. Briefly, on the experimental area, we chose three lines according to the method of Waddell [33]; each line is called a transect.

Table 1. Climatic conditions recorded in the region of Djelfa (south of Algeria) during 2016.

Month	Rainfall (mm)	Temperature (°C)	Relative Humidity (%)
January	6	7.8	67
February	24	7.5	71
March	30	8.4	68
April	30	13.8	53
May	7	18.6	44
June	1	22.8	36
July	6	27.1	35
August	4	25.6	39
September	18	20.5	51
October	13	17.8	55
November	24	9.4	71
December	23	6	87
Total	185		
Mean		15.4	56

2.2. Chemical Analysis

2.2.1. Oil Extraction

The oil content was determined according to the Soxhlet method (NF EN ISO 659). Ground seeds (10 g) were used for oil extraction during 6 h with 200 mL of cyclohexane. The solvent was removed in a rotary evaporator under low pressure at 50 °C.

2.2.2. Fatty Acid Composition

The fatty acid (FA) composition was determined, in triplicate, according to the ISO 12966-3 normative after the conversion of fatty acids into fatty acid methyl esters (FAME). The seeds were ground in an electric grinder. Five milliliters of tert-butylmethylether (TBME; ME0552. Scharlau) was mixed with 200 mg of ground sample. The mixture was filtered through a GHP filter with 0.45 µm pores. Then, 50 µL of 0.2 M trimethylsulfonium hydroxide (TMSH) in methanol (Macherey-Nagel) were mixed with 100 µL of the filtrate. The FAMEs were analyzed by gas chromatography coupled to flame ionization detection (Varian 3900). The GC was equipped with a CP-select CB for FAME column (50 m × 0.32 mm i.d., 0.25 µm film thickness), with helium as the carrier gas (1.2 mL/min). Split injector (1:100) and FID were maintained at 250 °C. The initial oven temperature was held at 185 °C for 40 min, increased to 250 °C at a rate of 15 °C/min, and then held there for 10 min.

2.2.3. Volatile Organic Compounds Identification

VOC emission analysis from pistachio materials was performed via SPME coupled with gas chromatography/mass spectrometry (GC6890/5973N Agilent, Les Ulis, France). Four fiber types were tested: polydimethylsiloxane (PDMS) 100 µm, polydimethylsiloxane/divinylbenzene PDMS/DVB (65 µm), polyacrylate (PA) 85 µm, and carboxen/polydimethylsiloxane CAR/PDMS (75 µm) (Sigma-Aldrich, Saint-Quentin-Fallavier, France). Prior to the analyses, the fibers were conditioned for 1 h in the injection port of the GC/MS, at 250, 250, 280, and 300 °C, respectively. The separation was achieved with a DB5MS capillary column (30 m × 0.25 mm; 0.25 µm film thickness) (Restek, Les Ulis, France) with He as the carrier gas (1.3 mL/min). The oven temperature was programmed from 45 °C (hold 3 min) to 150 °C (hold 2 min) at 8 °C/min and 250 °C (hold 10 min). The temperatures of the injector and quadrupole and ion sources were 250 °C, 150 °C, and 230 °C, respectively. The MS detector was run in electron impact mode with electron energy of 70 eV. The mass scan ranged from 35 to 400 m/z. Volatile organic compounds were identified by comparison of their retention indices, calculated by the use of a series of *n*-alkanes (C9–C16), with those reported in the literature. Further identification was carried out through comparison with library mass spectra (NIST Version 2.2).

3. Results and Discussion

Oil yield represented more than 40% of seed dry weight (DW). This yield was quite similar to the levels reported earlier [28,29] for seeds harvested in western Algeria under different weather conditions. Benhassaini et al. [34] observed higher oil content in seeds from the extreme west of Algeria. The yield we obtained can be considered high by comparison with those of oils from other oilseed crops (rapeseed and sunflower, for example), taking into account the fact that this species was not submitted to breeding programs to increase this trait [35]. Nevertheless, the climatic conditions observed in these studies were different from those reported in our study. This fact indicates that the oil yield from *Pistacia* seeds is dependent not only on environmental parameters but also on genetic factors [35,36]. Moreover, the oil extraction methods influence greatly the oil content [37]. The results of FA composition and oil content are presented in Table 2. In general, there was a predominance of monounsaturated fatty acids (MUFA) that represented more than 43% of total FA. The content of PUFA was slightly lower than that of SFA (Table 2). The major FAs were oleic (41%), linoleic (26.8%), and palmitic (26.7%) acids. Clearly, the oil was rich in unsaturated FAs (71.3%); the remaining FAs were saturated (28.7%). These levels are lower than those reported in other studies [28,29,34]. The differences could be due to genotypic and environmental [38–40] factors as well as to the methods used for the extraction and measurements of oil and fatty acids [35,41,42]. The ecotype seeds used in the cited studies were collected in 2006 in western Algeria, whereas the seeds used in our study were harvested in southern Algeria. Several factors like genotype, environmental conditions, type of soil, nutrients availability, year of cultivation could be involved in these differences [22,38,41,43]. In some species, oil content decreases with the increase of temperature [35,43,44]. These parameters could also contribute to modifying fatty acid composition. Indeed, Djelfa region is known as an arid zone, with only 185 mm of rainfall recorded in 2016 (Table 1). Unfortunately, we cannot compare our data with those of previous studies [28,29,34] in western Algeria, since climatic data are lacking in these reports.

Table 2. Oil content and fatty acid composition (%) of pistachio seed harvested in Djelfa (south of Algeria) in 2016 and results already published on pistachio harvested in different locations in Algeria.

Fatty Acids	Djelfa	Unkown [29]	Sfisef [28]	Tlemcen [34]
Saturated fatty acid (SFA)				
C16:0 (Palmitic acid)	26.7 ± 0.7	24.0	12.2	12.2
C18:0 (Stearic acid)	2.1 ± 0.0	1.8	2.4	2.4
C20:0 (Arachidic acid)	0.1 ± 0.1	-	0.1	0.1
Total SFA	28.8 ± 0.9	25.8	14.8	14.8
Monounsaturated fatty acid (MUFA)				
^{22}C16:1n-7 (Palmitoleic acid)	1.0 ± 0.0	1.2	1.8	1.8
C18:1n-9 (Oleic acid)	40.9 ± 0.2	46.0	54.2	54.2
C18:1n-7 (Vaccenic acid)	1.2 ± 0.0	-		-
C20:1n-9 (Eicosenoic acid)	0.2 ± 0.0	-		-
Total MUFA	43.4 ±0.2	47.2	55.9	55.9
Polyunsaturated fatty acid (PUFA)				
C18:2n-6 (Linoleic acid)	26.8 ± 0.7	27.4	28.8	28.8
C18:3n-3 (Linolenic acid)	1.1 ± 0.0	-	0.4	0.4
Total PUFA	27.9 ± 0.7	27.4	29.3	29.3
Total MUFA+PUFA	71.3 ± 0.9	74.6	85.2	85.2
Oil yield (%)	40.4 ± 2.2	45.0	40	39.8

The oil of *P. atlantica* was composed of 75% of unsaturated fatty acids (Table 2) and therefore appears to be a very healthy oil for humans, possibly contributing to lowering the risk of cardiovascular diseases. This composition is interesting, which is lower than those reported in Chia [43], and indicates a higher polyunsaturated FA level than in oils from sunflower, rapeseed, brown mustard, oak, soya, and Apiaceae species [22,26,35,45,46]. Oils rich in polyunsaturated fatty acids reduce the

risk of cardiovascular arterial or thrombotic hypertension, favor anti-inflammatory processes, and participate in the prevention of neurodegenerative diseases [20,21]. It has been reported that PUFA are cardioprotective, probably due to their anti-inflammatory, anti-arrhythmic, lipid-lowering, and antihypertensive activities [18]. PUFA are necessary for the proper functioning of the brain, eyes, and entire nervous system and to prevent the risk of cardiovascular diseases [18]. Palmitic (16:0) and stearic (18:0) acids are of great interest in the food industry [46]. Oleic acid is effective in lowering total cholesterol levels [18,47,48] and it is associated with a low risk of breast cancer [17]. Linoleic acid is an essential fatty acid and, therefore, it is important in human nutrition as a structural component of the phospholipid membranes of cells throughout the body [19]. Moreover, linoleic acid helps to prevent human diseases, particularly cardiovascular disease, and certain disorders like Alzheimer's disease [21]. Therefore, the oil of *P. atlantica* could be considered a healthy oil in relation to its fatty acid composition (Table 2).

The oil of Atlas Pistachio was aromatic. The results of the SPME analysis revealed indeed 43 volatile compounds (Table 3). The chromatograms obtained in the headspace study using PDMS/CAR fiber were similar to those obtained using PDMS/DVB fiber but presented more intense peaks, therefore identification was easier (Figure 1). The chromatograms obtained with PA and PDMS fibers presented fewer peaks with lower intensities. The identification was performed initially by comparison of the experimental mass spectra with those reported in the NIST library. The compounds detected were mainly monoterpenes ($C_{10}H_{16}$). Sesquiterpenes ($C_{15}H_{24}$) were detected (Table 3) in a smaller quantity (these molecules are less volatile). Some of these compounds including α-pinene, β-pinene, γ−terpinene terpinen-4-ol, D-germacrene, β-caryophyllene, bornyl acetate, and camphene were identified as major components of leaves' essential oils of Atlas pistachio depending on the region where the leaves were collected [30,31]. Moreover, these compounds have shown significant antimicrobial activity against Gram-positive bacterial strains compared to Gram-negative strains [8].

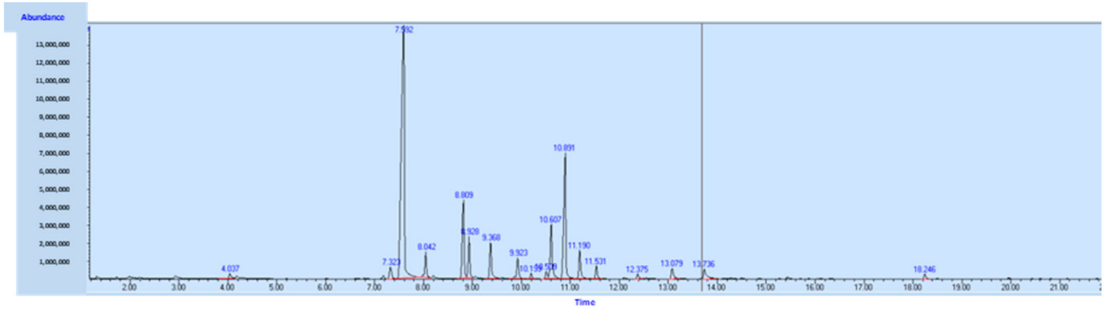
Table 3. Volatile organic compounds identified in the pistachio material headspace analysis.

t_R (min)	Compound	$RI_{litterature}$	$RI_{experimental}$
4.03	hexanal	799[a]	805
7.17	tricyclene	920[b]	922
7.32	α-thujene	925[a]	926
7.59	α-pinene	934[a]	935
8.04	camphene	946[a]	950
8.21	2,4(10)-thujadiene	961[c]	955
8.8	sabinene	973[a]	974
8.92	β-pinene	982[a]	978
9.36	β-myrcene	989[a]	992
9.86	α-phellandrene	1005[a]	1008
9.92	Δ3-carene	1013[a]	1010
10.19	α-terpinene	1034[a]	1019
10.51	p-cymene	1029[a]	1029
10.61	limonene	1031[a]	1032
10.89	Z-β-ocimene	1037[a]	1041
11.19	E-β-ocimene	1048[a]	1050
11.53	γ-terpinene	1061[a]	1061
12.1	cis-sabinene hydrate	1069[a]	1080
12.37	terpinolene	1091[a]	1088
12.55	fenchone	1094[f]	1094
12.6	$C_{10}H_{12}$		1096
13.07	nonanal	1104[a]	1111
13.73	allocimene	1130[a]	1133
14.48	trans-verbenol	1148[b]	1158
14.85	pinocarvone	1162[c]	1171

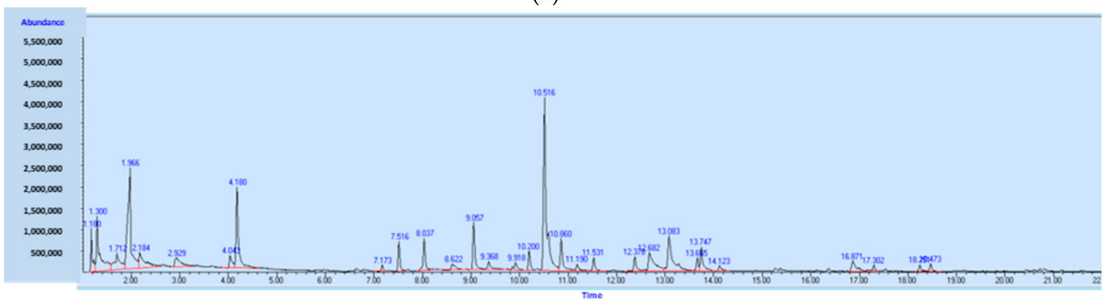
Table 3. *Cont.*

t_R (min)	Compound	$RI_{litterature}$	$RI_{experimental}$
15.27	borneol	1173[(a)]	1185
15.44	terpinen-4-ol	1181[(b)]	1190
16.32	verbenone	1211[(c)]	1221
16.36	$C_{10}H_{14}O$		1223
18.24	bornyl acetate	1286[(d)]	1290
19.98	α-terpinyl acetate	1348[(e)]	1355
20.44	α-ylangene	1368[(e)]	1373
20.6	α-copaene	1376[(c)]	1379
20.82	β-bourbonene	1392[(b)]	1387
21.78	β-caryophyllene	1437[(a)]	1425
23.18	γ-muurolene	1486[(a)]	1481
23.33	D-germacrene	1480[(c)]	1487
23.77	α-muurolene	1501[(c)]	1505
24.12	γ-cadinene	1521[(e)]	1519
24.24	cardina-1(10),4-diene	1531[(e)]	1524
34.00	palmitic acid		
36.10	oleic acid		

RI: Retention indice (a): Kondjoyan and Berdagué [49], (b): Dwivedy et al. [50], (c): Morshedloo et al. [51], (d): Araujo et al. [52], (e): Stewart et al. [53], (f): Bilia et al. [54].



(**a**)



(**b**)

Figure 1. Chromatograms resulting from solid-phase microextraction (SPME)–GC–MS analysis of the crushed seeds headspace using two different fiber coatings: (**a**) carboxen/polydimethylsiloxane (PDMS) (75 µm) and (**b**) PDMS/divinylbenzene (DVB) (65 µm) (T, 23 °C; $t_{adsorption}$, 30 min).

Several works have been performed to investigate alternative medicines for natural therapies (See review [27]). This interest in natural drugs has been highlighted by the World Health Organization, suggesting the importance of ethnomedicines and natural drugs [15,25,42,55]. *P. atlantica* is used in different regions as a traditional remedy [27]. Table 4 presents some activities of different extracts of *P. atlantica*, *Pistacia khinjuk*, *Pistacia lentiscus*, and *Pistacia vera*.

Table 4. Activities of extracts from *Pistacia atlantica* and *other* Pistacia species.

Species	Plant Part	Extract	Component	Activity	References
P. atlantica	Hull of fruit	Essential oil	Myrcene, α-Pinene, Limonene and α-Humulene	Antibacterial	Rezaie et al. [56]
	Leaves	Essential oil, resin		Antibacterial	Hamelian et al. [57]
		gum		Reduce growth and aflatoxin production of *Aspergillus parasiticus*	Khodavaisy et al. [58]
	gum, fruit and leaves	Essential oil		fumigant activity against *Callosobruchus maculatus*	Pourya et al. [14]
	Leaves			antifungal *Geotrichum candidum*	Talibi et al. [11]
	leaves			Healing effect	Tanideh et al. [59]
	Hull	Essential oil		gastroprotective anti-*Helicobacter pylori* anti-*Staphylococcus*	Taghizadeh et al. [60]
	seed	Oil and fatty acids		Decrease of colonic mucosal blood flow	Shimizu et al. [61]
	Fresh unripe fruits			Anti-breast cancer	Rezaei et al. [62]
	Seed	Oil and fatty acids		Anticholesterolemic	Nazifi et al. [63]
Pistacia lentiscus		Gum		Management of inflammatory bowel diseases	Kaliora et al. [64]
	fruits	Lipids		Anti-inflammatory	Chaabani et al. [23]
	Leaves	Essential oil	α-Pinene	Antibacterial	Derwich et al. [65]
Pistacia khinjuk	Leaves			Antihyperlipidemic	Kamal et al. [66]
		Gum		Antibacterial	Fattahi et al. [67]
Pistacia vera	Hull	Essential oil		Antibacterial	Smeriglio et al. [68]

P. atlantica was found to be rich in lipids, fibers, and proteins with antioxidant activities. This plant is used to treat gastrointestinal disorders and as an antiseptic, laxative, diuretic, and carminative drug [69,70]. In North Africa, Atlas Pistachio fruit or leaves decoctions are used traditionally by the local population to treat eye infection or diarrhea [69]. Recent studies emphasized other properties such as antioxidant, antimicrobial, antidiabetic, and antitumor activities [7,57,69,71]. Several studies have been devoted to the effects of bioactives from *Pistacia* species, including Atlas Pistachio, including gastroprotective and anti-*H. pylori* activities [4,60]. Pourya et al. [14] found that the essential oil of *P. atlantica* can be used as a fumigant and repellent due to contact toxicity against *C. maculatus*. Hence, our data can be further exploited to develop a management strategy against *C. maculatus*. This insect, known as the cowpea weevil or cowpea seed beetle, causes important damage on stored seeds. Recent works studied *P. atlantica* essential oil as an antibacterial and insecticidal agent. It was reported that the protective effect of *P. atlantica* essential oil against gastric ulcer and its activity against *H. pylori* and other pathogens was due to the presence of α-pinene. In our study, α-pinene was found among the 43 volatile compounds (Table 3). These results correspond to those reported in studies on the characterization of the essential oil of *P. atlantica* [12,72]. This fact may, probably, explain why peoples who consume seeds or leaves decoctions of *P. atlantica* are less sensitive or less exposed to gastrointestinal disorders [30,69]. It appears, therefore, that the oil of *P. atlantica* that contains VOC could be considered a healthy oil and be proposed for human alimentation.

4. Conclusions

Our study revealed that the seeds of Pistachio of Atlas contain a high amount of oil, which is rich in mono- and polyunsaturated fatty acids. SPME analysis allowed detecting 43 volatile organic compounds which may contribute not only to the smell of this oil but also to its conservation. α-pinene was among the volatile organic compounds found in our study. This molecule seems to be active

against a large panel of bacteria and fungi as well as insects. These characteristics suggest that this oil is very healthy for humans and should be included in the diet. More investigations are needed to study the variability of oil content and oil fatty acid composition of different ecotypes of *P. atlantica*. VOC quantification may help to better characterize and define the possible use this oil.

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