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Total Tocopherol Content and Antioxidant Activity of Fruit Oil from *Pistacia atlantica* Desf. Growing Wild in Algeria

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ABSTRACT

Pistacia atlantica fruit oil has been used for a long time by local population for culinary and medicinal purposes. In this study, the chemical properties and total tocopherol content were determined in twelve samples of P. atlantica fruit oil at three stages of maturation (immature, intermediate maturity and mature) collected in three different sites from the region of Laghouat. Their antioxidant activities against free radical (2,2-Diphenyl-1-Picrylhydrazyl) (DPPH) were also investigated. The results indicated a significant difference between the oil of mature fruits (green and black) and the immature ones (light red) which were distinguished by richness in tocopherols. Moreover, their antioxidant activity was the highest. The oil from fruits of intermediate maturity (dark red) seems to combine these properties with those of the mature group in particular significant yield. Such data emphasize the value of this oil which needs further investigations.

Keywords: *Pistacia atlantica*, Fruits, Oil, Tocopherol, Antioxidant, Maturation stage

INTRODUCTION

Algeria, the largest country of Africa, holds very much diversified flora. This natural richness remains insufficiently known. One of the botanical species which has not delivered yet all its mysteries is *Pistacia atlantica* Desf. (Atlas pistachio or elbetoum in local Arabic). This plant which belongs to the *Anacardiaceae* family is a big coniferous, dioecious, powerful tree, and it could reach 15 m of height [1]. *P. atlantica* grows particularly in semi dry areas. In Algeria, it is notably located in the Saharan Atlas, in the regions of Djelfa, Laghouat and Ghardaia [2]. Fruits of this plant are widely consumed by the local population as nutriment. The oil which is extracted from the fruit is usually used in the traditional medicine against several diseases [3].

The antioxidant activity of the extracts of plants arouses a particular interest. Such interest is too important that world knows a recrudescence of diseases in relation with oxidation processes like the degenerative and inflammatory diseases and the atherosclerosis [4,5]. Indeed, during oxidative stress, large amounts of Reactive Oxygen Species (ROS) are produced. These species can attack biological molecules such as proteins, nucleic acids and lipids [6]. Furthermore, ROS are directly involved in the processes of cellular aging [7]. In such situation, the endogenous antioxidants are not sufficient to reduce the ROS which require exogenous antioxidants. The natural antioxidants take place in the foreground since they generally have fewer undesirable effects with regard to those of synthesis.

Several works had revealed that plants constitute a major source of natural exogenous antioxidants [8,9]. Among these plants, *P. atlantica* has a great value because of the antioxidant activity of its extracts [10,11], the composition of its fruit oil [12-15], its flavonoids content [16] and its anti-cancerous activities [17]. All these virtues explain its diverse uses by local population [2,18]. Very few data are available about the variation of the composition of *P. atlantica* fruit oil, as well as their antioxidant activity according to the degree of maturation.

The aim of this work was the study of the chemical characteristics and total tocopherol content of the oil extracted from *P. atlantica* fruits, stemming from several sites in Laghouat, according to the degree of maturation. We also analyzed the antioxidant power of theses extracts using the (2,2-Diphenyl-1-Picrylhydrazyl) (DPPH) test. Such approach could supply useful and exploitable information for possible applications.

MATERIALS AND METHODS

Plant material

Pistacia atlantica fruits were collected in August and September in three different sites from the region of Laghouat. After air-drying in the shade at room temperature for one week, twelve samples were used for this study. These samples were divided into three groups according to the maturation degree revealed by the skin color.

It is important to note that fruits maturation does not take place in an identical way for the same tree. Indeed, there are fruits which finish their maturation prematurely and others later. In that case it is not surprising to find mature fruits at the beginning of August and immature fruits at the end of September. The characteristics of samples are shown in Table 1.

Table 1: Characteristics of *Pistacia atlantica* fruits samples

Sample	Color of the skin	Degree of maturation	Month of harvest	Site of harvest
G1[a]	Green	Mature	August	Site 1
DR1[a]	Dark red	Intermediate maturity	August	Site 1
LR1[a]	Light red	Immature	August	Site 1
G2[a]	Green	Mature	September	Site 1
DR2[a]	Dark red	Intermediate maturity	September	Site 1
LR2[a]	Light red	Immature	September	Site 1
G3[b]	Green	Mature	September	Site 2
LR3[b]	Light red	Immature	September	Site 2
G4	Green	Mature	September	Site 3
LR4	Light red	Immature	September	Site 3
B1	Black	Mature	September	Site 3
B2	Black	Mature	September	Site 3

a and b mean that the fruits were collected from the same tree in sites 1 and 2 respectively. Site 1: 2°59’32,58"E, 33°30’37,07"N, 873 m; Site 2: 2°39’44,19"E, 33°41’23,50"N, 836 m; Site 3: 3°21’16,07"E, 33°09’17,84"N, 736 m

Reagents and standards

All chemicals were purchased from Sigma (USA), Aldrich (Milwaukee, USA), Fluka Chemie (Buchs, Switzerland) and Merck (Germany).

Oil extraction

The seeds (100 g) were milled into powder using a manual mill and extracted with hexane (150 ml) by stirring at room temperature for 48 h. Samples were then dehydrated with anhydrous sodium sulfate and filtered. The solvent was evaporated under reduced pressure using a rotary evaporator at 40°C. The dried crude oils were stored at +4°C until use.

Determination of chemical properties of the oils

Acid value, saponification value and the iodine value were determined according to the procedure described by the AFNOR (Association Française de Normalisation) i.e., NF T60-204 for acid value, NF T60-206 for saponification value and NF T60-203 for iodine value [19].

Total tocopherol content determination

The total tocopherol content was determined according to the colorimetric method of Emmerie-Engel with minor modifications [20]. Briefly, 0.1 g oil samples were diluted in butanol. Then, 1 ml of 0.4% 1,10-phenantroline and 0. 5 ml of 0.12% $FeCl_3$ (prepared in ethanol) were added to 1 ml of each dilution. After 5 min of incubation at room temperature, the absorbance was measured at 510 nm against a blank. A calibration curve was realized with known concentrations of commercial vitamin E in the same conditions. The results are expressed as mg α-tocopherol equivalents by 100 g of oil. Experiments were carried out in triplicate.

Free radical (DPPH) scavenging activity

The DPPH scavenging activity of the different samples was assessed as it was described by Brand-Williams et al. [21], with little modification. Succinctly, 1 ml of sample dilutions in butanol was added to 1 ml of 250 µM solution of DPPH prepared in methanol. After 30 min of incubation at room temperature, the absorbance was measured at 517 nm against a blank. The normal purple color of DPPH will turn into yellow when it is reduced by an antioxidant in dose dependent manner [22]. Ethanol (1 ml) in place of the oil sample was used as control. The percent inhibition activity was calculated using the following formula:

$$I(\%)=[(A_{control}-A_{sample})/A_{control}] \times 100$$

Where, $A_{control}$ the absorbance of the control reaction (DPPH with only ethanol) and A_{sample} is the absorbance in the présence of the extract sample. The concentration of the extracts providing 50% inhibition (EC_{50}) was calculated from the graph plotting inhibition percentages against extract concentration. The commercial vitamin E was used as positive control.

Statistical analysais

The Student's test was used for the statistical comparisons. Differences were considered to be significant at $P<0.05$, $P<0.01$ or $P<0.001$.

RESULTS AND DISCUSSION

Oil content and its chemical properties

Oil obtained from *Pistacia atlantica* mature fruits have a clear yellow color with a pleasant smell, whereas, oil of immature fruits gives a green color with a very pleasant smell. Results of oil content and chemical properties are shown in Table 2. The oil content varies considerably according mainly to the maturation degree. Indeed, the highest yields (from 27.7 to 46.06%) are obtained with mature fruits (green or black) compared to immature ones ($P<0.001$). Fruits of intermediate maturity have medium oil content (20.08 and 34.98%). Our results are similar to those reported by the literature for *Pistacia lentiscus* [23,24]. It seems from our results that the accumulation of oil in the *P. atlantica* fruits occurs during maturation.

Table 2: Oil content and chemical characterization

Sample	Oil (% w/w)	AV (mg KOH/g)	SV (mg KOH/g)	IV (Wijs)
G1	27.7	6.73	212.8	81.28
DR1	20.08	5.04	204.4	90.81
LR1	16.6	8.97	201.6	102.9
G2	37.7	4.2	208.6	74.93
DR2	34.98	5.33	194.6	92.08
LR2	16.77	11.22	201.6	94.62
G3	37.75	15.15	201.6	70.49
LR3	6.99	14.03	179.2	90.17
G4	41.72	4.49	180.6	73.03
LR4	14.95	10.66	193.2	87.63
B1	46.06	7.57	212.8	76.84
B2	35.7	21.32	193.2	99.95
Mean of immature samples	13.83 ± 4.63**	11.22 ± 2.10	193.90 ± 10.57	93.83 ± 6.70*
Mean of intermediate samples	27.53 ± 10.54	5.19 ± 0.21	199.50 ± 6.93	91.45 ± 0.90
Mean of mature samples	37.77 ± 6.16	9.91 ± 6.86	201.60 ± 12.74	79.42 ± 10.70

AV: Acid Value; SV: Saponification Value; IV iodine value; **P<0.001; *P<0.05 (compared with mature samples)

The high acid value found for the most part of samples (Table 2) is perhaps due to poor conservation of the fruits [12]. The saponification values (Table 2) indicate that oils contain essentially fatty acids with 16-18 carbon atoms [12]. For this parameter, our results are comparable to other useful oils such as corn oil (187-195 mg KOH/g), peanut oil (187-196 mg KOH/g), palm oil (190-209 mg KOH/g), and olive oil (182-201 mg KOH/g) [25,26].

The iodine value shown in Table 2 is significantly higher in immature fruit oil (mean value 93.82 ± 6.70) in comparison with mature fruit oil (mean value 79.42 ± 10.70, P<0.05). This result indicates the presence of many unsaturated bonds, therefore a high level of unsaturated fatty acids in the immature samples. Charef et al. [23] reported similar results for *P. lentiscus* seed oil. We notice with interest the relatively high iodine value for the samples of intermediate maturity (mean value 91.45 ± 0.90).

We had previously reported that *P. atlantica* fruit oil contained mainly palmitic, oleic and linoleic acids. The proportion of unsaturated fatty acids (oleic and linoleic acids) were slightly higher in the oil of immature fruits [27]. These results are in perfect agreement with those of saponification and iodide values. Generally it seems that the iodine value indicating the degree of unsaturation decreases with the fruit maturation.

Total tocopherol content

The results of total tocopherol content are exposed in Table 3. It was in the range of 51.35-170.08 mg/100 g of oil. The oil extracted from the immature fruits contained a substantial amount of total tocopherols (mean value 140.61 ± 23.07 mg/100 g) than the one of the mature fruits (mean value 86.14 ± 24.44 mg/100 g, P<0.001). Samples with intermediate maturity showed practically an intermediate value (mean value 115.15 ± 17.78 mg/100 g).

Table 3 : Total tocopherols content (mg/100 g oil)

Sample	Total tocopherols
G1	123.01 ± 21.22
DR1	127.72 ± 16.79
LR1	134.85 ± 10.52
G2	97.83 ± 8.36
DR2	102.58 ± 1.37
LR2	143.12
G3	51.35 ± 3.74
LR3	170.08
G4	71.13 ± 1.25
LR4	114.37 ± 7.49
B1	81.56 ± 0.69
B2	91.94 ± 2.18
Mean of immature samples	140.61 ± 23.07**
Mean of intermediate samples	115.15 ± 17.78

P. atlantica fruit oil appears to be richer in tocopherols (particularly in the immature stage) than other food vegetable oils such as the sunflower oil (67 mg/100 g) and the olive oil (20 mg /100 g) [28]. This richness would supply to the oil beneficial properties. Indeed, Tocopherols, notably α-tocopherol, not only they have a protective effect on cells against oxidative stress, inflammatory response, lipid peroxidation and cancer but contribute to the natural protection and conservation of the oil against oxidative deterioration [29-32]. We should not neglect the anticancer, antidiabetic and cardioprotective effect of tocotrienols which are demonstrated now clearly [33]. Thus, the study of these compounds in *P. atlantica* fruit oil is of a great interest.

Free radical (DPPH) scavenging activity

The graph plotting inhibition percentages against the concentrations of oil samples in the DPPH solution provides a direct linear relationships with a determination factor R^2>0.995. This linear equation allows us to calculate the concentration of oil providing 50% inhibition of the free radical (EC_{50}). Commercial vitamin E (α-Toc) was used as standard. Results are grouped in Table 4. EC_{50} values for all samples were ranged from 17.1-51.3 mg/ml. Immature fruit oil had a raised antioxidant activity (mean value of EC_{50}: 26.18 ± 7.30 mg/ml) than mature fruit oil (mean value of EC_{50}: 40.13 ± 8.61 mg/ml, P<0.05). EC_{50} of samples with intermediate maturity was between these two groups (mean value: 32.30 ± 1.27 mg/ml). Oil samples were less active compared to the standard used which had a very strong anti DPPH activity (EC_{50}: 0.028 mg/ml).

To the author's knowledge, there are no published works about antioxidant activity of oils extracted from *P. atlantica* fruits. Most of works focused on the assessment of antioxidant power in leaves extract which was judged appreciable [10,11,34].

Table 4: Anti DPPH activity

Samples	Commercial vitaminé E	G1	DR1	LR1	G2	DR2	LR2	G3	LR3	G4	LR4	B1	B2
EC_{50} (mg/ml)	0.028	31.9	33.2	28	33.5	31.4	24.9	51.3	17.1	44.6	34.7	47.3	32.2
Mean of EC_{50} (mg/ml)	-	Immature samples				Intermediate maturity samples				Mature samples			
		26.18 ± 7.30*				32.30 ± 1.27				40.13 ± 8.61			

EC_{50} concentration that produces 50% scavenging of the DPPH. *P<0.05 (compared with mature samples)

In the aim to find possible relationships between total tocopherol content and the antioxidant activity, the graphs plotting EC_{50} (for all samples) against total tocopherol content were drawn. Results are presented in Figure 1. Taking into account the inversely proportional relationships between antioxidant capacity and EC_{50}, this later correlates well with total tocopherol content (determination factor R^2=0.865) (Figure 1).

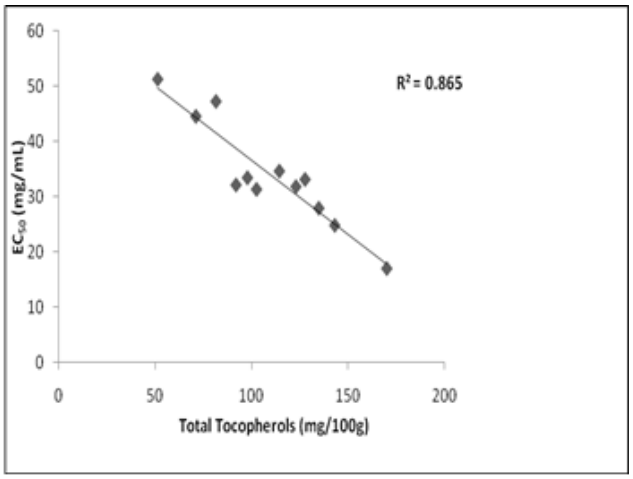

Figure 1: Relationships between total tocopherol content and antioxidant activity expressed as EC_{50} values

CONCLUSION

In this study, we determined some chemical properties, total tocopherol content and the antioxidant activity against DPPH of *P. atlantica* fruit oil in three stages of maturation; immature, intermediate maturity and mature. In light of our results, almost all these parameters could constitute a specific character of maturation degree. It should be noted that the oil of the immature fruits (light red) had high level of tocopherols as it presented a powerful antioxidant activity. Thus, it represents a valuable source of natural antioxidants. However, their yield was the lowest. Oil of fruits with intermediate maturity (dark red) showed intermediate characteristics. With a relatively high yield and richness in tocopherols associated to an appreciable antioxidant activity, this oil must be extensively studied. Finally, we can say that our work have brought very interesting and original preliminary results supporting that *P. atlantica* fruit oil before full maturity might be a healthy diet and a valuable source of non-toxic natural antioxidants. Further studies are needed for the identification of specific compounds responsible for the antioxidant activity.

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