J. Mater. Environ. Sci. 5 (1) (2014) 199-206
ISSN : 2028-2508
CODEN: JMESCN

Chemical composition, mineral contents and antioxidant activity of fruits of *Pistacia lentiscus* L. from Eastern Morocco

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Received 29 May 2013, Revised 24 July 2013, Accepted 24 July 2013
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Abstract
The aim of this work is the valorization of *Pistacia lentiscus* L. by chemical characterization, mineral composition, and study of the antioxidant activity of essential oil. The hydrodistillation of the fruits of this tree provides essential oil (0.56 %). 3-carene (54.10 %) and α- pinene (7.64 %) were found to be the major components. The essential oils of leaves and fruits were subjected to screening for their possible antioxidant activities by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH). The results showed that the essential oils of leaves and fruits of *P.lentiscus* collected from Saidia (Eastern Morocco) were found to be less efficient in radical scavenging with an IC_{50} values of 23.79 µl/ml and 29.63 µl/ml respectively, by comparison with the essential oil of leaves from Taforalt(Eastern Morocco) (IC_{50}=12,8 µl/ml). Mineral contents of fruits were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES). The highest levels of K and Cu were found in fruits of *P.lentiscus* from Saidia (Eastern Morocco).

Keywords: Pistacia lentiscus L., Essential oil analysis; 3-Carene, Mineral composition, Antioxidant activity.

1. Introduction
In the world, up to 500000 plant species are reported [1]. Few of them are used in herbal medicine. The medicinal properties of plants come from the presence of bioactive agents in their extracts. The Mediterranean region is characterized by heterogeneous soil and climatic conditions that have produced more than 10000 medicinal and aromatic plant species with diverse properties worthy of further investigation [2]. The systematic investigation of such plants will help to define their precise pharmacological properties and to determine their value as functional foods and as a source of nutraceutical compounds such as novel antioxidants [3, 4].
Pistacia lentiscus L. is an evergreen shrub of the family Anarcadiaceae. This dioecious species can reach 3 m in height and grows in many Mediterranean countries [5].
The aerial parts of *P. lentiscus* L. has traditionally been used in the treatment of hypertension and possesses stimulant and diuretic properties [6]. The resin part of this plant known as mastic resin and plant called as mastic tree [7,8], it has a great medicinal value and already been used in traditional system of medicines like Unani and Ayurveda system [9] and used as chewing gum, against lip-dryness, some stomach diseases and antiseptic for respiratory system [10-12].
Essential oils have been traditionally used for the treatment of infections and diseases all over the world for centuries [13].
Multiple studies have been reported on the chemical composition of essential oil of *Pistacia lentiscus* belonging to different regions in the world [11, 14-18].
The essential oil of leaves of *Pistacia* species has been the object of several studies on antioxidant activity [19-21] and a lot of studies have been reported on the antioxidant property of *P. lentiscus* [22].
The essential oil of *Pistacia lentiscus* collected at flowering stage which contains high monoterpene hydrocarbon fraction, showed highest free radical scavenging activity and antioxidant capacity [19, 23-24].

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones. Data from both scientific reports and laboratory studies show that plants contain a large variety of substances that posses antioxidant activities [25].
Several studies have been carried out on the organic constituents of the medicinal plants, but limited studies on mineral contents of condiments were made [26-29].
Throughout the world, there is increasing interest in the importance of dietary minerals in the prevention of several diseases.
The aim of the present study, which is carried out for the first time for *P.lentiscus* grown in Eastern Morocco, is to acquire valuable information about the chemical composition of the fruits essential oil, and analyze the difference in trace elements levels in the leaves and fruits, in the end, this study attempted to evaluate the antioxidant activity of the leaves and fruits oils of this medicinal plant.

2. Materials and methods and isolation procedure

2.1. Plant material

The aerial parts of *P. lentiscus* L. (Anacardiaceae) were collected from Eastern Morocco: Saïdia (35°05'N, 02°14'W), and Taforalt (34°48'26''N, 2°24'48''W). Before extraction, plants were dried in the shade for a week.

2.2. Essential oil isolation

The leaves and fruits of *P. lentiscus* L. were hydrodistilled on a Clevenger type apparatus for three hours, and the colorless and yellowish essential oils were obtained with yields 0.15 % and 0.56 %, respectively. The samples oils were dried using anhydrous sodium sulphate and kept in sterile sample tubes in refrigerator.

2.3. Essential oil analysis

The oil was analysed using a Hewlett Packard 6890 gas chromatography GC equipped with a Hewlett Packard 6890 mass selective detector and a HP-5 MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). The column was temperature programmed as follows: 40°C for 5 min, and then the temperature was increased to 280°C at a rate of 10°C/min. Helium was used as carrier gas (20 ml/min); the injection volume was 0.1µl in the splitless mode.
The MS operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass unit were monitored from 30 to 450 m/z.
Identification of components in the oil was based on retention indices relatives to n alkanes and computer matching with the WILLEY 275 library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature [30].

2.4. Mineral analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES)

150 mg of the plant sample was conserved with 2 ml HNO_3 acid (70%) mixture in a Teflon beaker. The sample was incinerated at 110°C. 0.5 ml of Hydrofluoric acid (HF) was added and the covered beaker placed on a sand bath. The sample mixture was heated until a clear solution was obtained. After removing the cover, the mixture was evaporated until drying. 2 ml of HCl acid was added. The residue was extracted by 25 ml 2M HCl. The concentrations were determined via an ICP-AES analysis.

2.5. Antioxidant activity

The free radical-scavenging activities of essential oil were measured using DPPH as described by Burits & Bucar [31]. Various concentrations of the oil (2-10µl/ml) in methanol were added to 2.4ml of a DPPH radical solution in methanol (the final concentration of DPPH was 4µg/ml) the mixture was strongly shaken and left to stand at room temperature for 30min in the dark.
The absorbance was measured at 517nm against a blank. Inhibition of the free radical, DPPH in percent I (%) was calculated according to the formula:

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

where A control is the absorbance of the control reaction. A sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against sample concentration.
Tests were carried out in triplicate. Ascorbic acid was used as a positive control.

3. Results and discussion

3.1. Chemical composition of the essential oils

The essential oils were extracted by the hydro-distillation of the dried parts from leaves and fruits of *P.lentiscus* from Eastern Morocco and the oils were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

The identified combinations in essential oil, time retention (Rt) and quantitative percentage of the compounds from leaves and fruits are presented in Table 1.

Table1: Essential oil components of leaves and fruits of *P. lentiscus.*

N°	Compounds	KI	Rt (min)	Leaves (Saidia)[a]	Fruits (Saidia)
				Percentage (%)	Percentage (%)
	Monoterpene hydrocarbons			**44.99**	**63.34**
1	α-pinene	934	9.17	13.94	7.64
2	Camphene	948	9.49	3.10	0.31
3	Sabinene	974	10.06	0.87	-
4	β-pinene	977	10.11	4.45	-
5	Myrcene	991	10.43	1.68	-
6	α-phellandrene	1030	11.16	-	1.21
7	α-terpinene	1017	10.93	0.81	0.08
8	p-cymene	1026	11.09	1.22	-
9	Limonene	1031	11.18	18.92	-
10	3-carene	978	10.14	-	54.10
	Oxygenated monoterpenes			**13.25**	**0.00**
11	linalool	1100	12.45	0.41	-
12	Endo-borneol	1172	13.60	0.49	-
13	α-terpineol	1195	13.98	6.78	-
14	terpinen-4-ol	1183	13.78	5.57	-
	Sesquiterpene hydrocarbons			**16.18**	**2.72**
15	Gurjunene	1445	17.44	-	0.25
16	β-caryophyllene	1436	17.33	6.93	0.68
17	α-cubebene	1387	16.71	-	1.11
18	Muurolene	1516	18.31	1.49	-
19	γ-Cadinene	1657	20.03	2.68	-
20	δ-Cadinene	1538	18.59	2.65	-
21	germacrene-D	1445	17.44	1.65	-
22	α-copaene	1661	20.07	0.78	0.10
23	α-cadinene	2668	27.81	-	0.58
	Oxygenated sesquiterpenes			**0.00**	**0.00**
	Total identified			**74.42**	**66.06**
	Yield (%)			**0.15**	**0.56**

[a]Chemical composition of the essential oil of the leaves of *P. Lentiscus* L.[32]
KI, kovat's indices
RT (min), retention time

The fruits oil was characterized by abundance monoterpene hydrocarbons (63.34 %). The leaves oil contained monoterpenes hydrocarbons (44.99 %), oxygen-containing monoterpenes (13.25 %) and sesquiterpenes (16.18 %) [32].

The main constituents of the fruits oil: 3-carene (54.10%) and α-pinene (7.64%) and the leaves oil: α-pinène (13.94%), limonene (18.92%), β-caryophyllene (6.93 %), α-terpineol (6.78%) and terpinen-4-ol (5.57 %).

Significant qualitative differences were found in terms of chemical composition in the oils obtained from different parts of *P. lentiscus* (Table 1). The leaves oil was rich in monoterpenes and sesquiterpenes, but in fruits, sesquiterpenes have been detected in small quantities and in trace amounts.
The quality of the materials forming essential oil had some differences and similarities with the cases reported in other studies.
The major constituents of the fruits essential oils of *P. lentiscus* from Spain [33] and Australia [34] were: myrcene (72 %, 39 %), α-pinene (10 %, 28 %) and limonene (7 %, 11 %) respectively.
For further comparison, leaves and fruits of *P. lentiscus* from Israel gave essential oils which contained beside the usual monoterpenes α-terpineol and terpinen-4-ol, several acyclic compounds mainly represented by ethyl linolenate and/or ethyl hexadecanoate [35].
Other study has reported the chemical composition of the fruits essential oil of *P. lentiscus* from Tuscany (Italy) which was determined by GC/MS. The leaves oil contained α-pinene (16.1–25.3 %), limonene (6.6–12.3 %), terpinen-4-ol (7.6–12.7 %) and germacrene-D (9.6–14.3 %) as major components, while the fruit oil contained α-pinene (7.5–11.2 %), myrcene (68.2–71.0 %) and limonene (9.6–19.7 %) as major constituents [36].
A scientific study of the chemical composition of the essential oil of fresh leaves and semi-ripe fruits of *P.lentiscus* L. of the Holy Land and the Sinai X. were investigated. The leaves oil was found to be rich in α-terpineol (13.02 %), ethyl hexadecanoate (10.36 %), ethyl linolenate (11.30 %) and some unidentified sesquiterpene alcohols (15.90 %), while the fruit oil was found to be rich in terpinen-4-ol (13.07 %), α-terpineol (13.62 %) and some unidentified sesquiterpene alcohols (13.20 %) [35].
Mans H. Boelens et al [33] studied the essential oils isolated by hydrodistillation from leaves, and unripe and ripe fruits of *P. lentiscus* L. The main constituents of the unripe-fruits oil: α-pinene (22 %) and β-myrcene (54 %), and of the ripe-fruits oil: α-pinene (11 %) and β-myrcene (72 %).
The results of our study could be of interest for further phytochemical investigation of *P. lentiscus* from Eastern Morocco.

3.2. Mineral composition of leaves and fruits of Pistacia lentiscus L.
The objective of this study was to quantify the content of various elements that might be responsible for some properties of *P. lentiscus.* Investigated elements were chosen (Zn, Fe, K, Cu, Mn, Ca, Mg, Se, Cd, Cr, Pb, La, V, and Li) according to their role and importance in many biological mechanisms. Quantitative determinations were made of the mentioned elements in fruits and leaves of *P. lentiscus* grown in Eastern Morocco (Saidia). The composition in major and minor minerals of the fruits and leaves of *P. lentiscus* is detailed in Table 2.
The variation observed in the mineral composition of *P. lentiscus* (fruits and leaves) in the present study (Table 2 and Figs. 1 and 2) is trivial and could be attributed to the part of the plant studied.

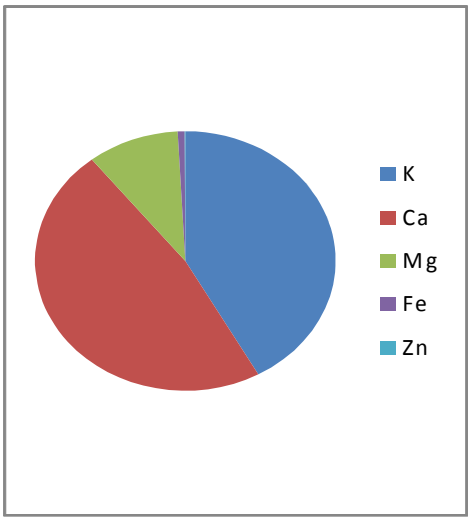

Figure 1: Percentage mineral compounds of leaves of *P. lentiscus.*

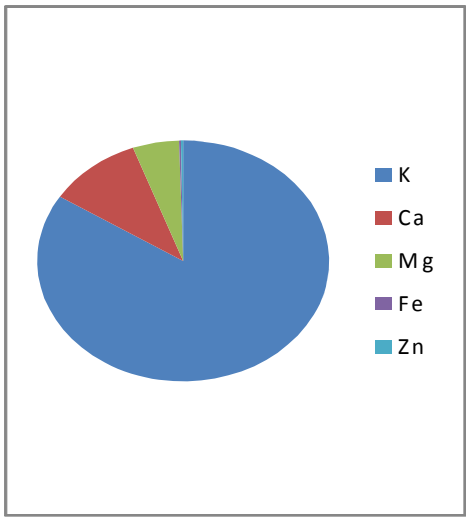

Figure 2: Percentage mineral compounds of fruits of *P. lentiscus.*

Table 2: Mineral contents of *P. lentiscus* from Saidia

Mineral Compounds	Leaves[a] (mg/Kg)	Fruits (mg/Kg)
Cd	6.45	3 .96
Cr	5.12	2.38
Cu	33.55	80.09
Pb	25.81	11.5
Zn	230.36	168.11
Mn	226.49	171.28
Se	17.42	12.29
La	14.09	4.88
Li	41.68	9.59
V	24.39	11.46
Ca	144400	36900
Fe	2300	800
K	127800	297800
Mg	30000	18200

[a] Mineral composition of the leaves of *P. lentiscus* from Saidia [32].

The concentration of K ranges from 127800 mg/kg to 297800 mg/kg in *P.lentiscus* fruits and leaves from Saidia respectively. The *P.lentiscus* fruits recorded high accumulation value of potassium. In general, the fruits are very rich in K, as all the plants studied, and the K accumulates in fruits.

The level of Ca in the leaves of *P.lentiscus* in this work was found to be higher than this in the fruits, Calcium is the major component of bone and assists in teeth development [37], and while high intake of Ca may damage the heart muscle and the thyroid gland. The leaves are always rich in Ca than fruits, and the difference in Ca content in fruits and leaves is very pronounced (144400 mg/Kg in leaves against 36900 mg/Kg in fruits).

Magnesium is not only essential, but it is also a constitutive element of chlorophyll, so that its highest concentration was found in leaves. The average concentration of Magnesium in the *P.lentiscus* leaves is 30000 mg/Kg against 18200 mg/kg in *P.lentiscus* fruits.

Zinc is an essential mineral that is found in almost every cell. It stimulates the activity of approximately 100 enzymes [38]. Zinc supports a healthy immune system and is needed for wound healing, the sense of taste and smell, and for DNA synthesis [39-41]. Antioxidant role of Zn [42] are well known. It also prevents oxidation of lipids and proteins initiated by heavy metals particularly by Fe^{2+} [43]. A deficiency can cause poor reproductive performance, growth retardation, abnormal formation of bone, cartilage, an impaired glucose tolerance, bleeding disorder [44]. Zn concentrations were significantly different at *P.lentiscus* fruits and *P.lentiscus* leaves. The Zn concentration in leaves was found to be higher than in the fruits (230.36 mg/kg in leaves against 168.11 mg/kg in fruits).

Iron is the one of the most essential elements needed by plants as well as human beings. Though it is an essential element, excess intake can lead to iron toxicity and can damage lipids and proteins [45-46].

Significant differences in the concentration of Fe in leaves and fruits, iron concentration in the leaves was found to be higher than that of the fruits, the leaves are still iron-rich as fruits with a content of about 2300 mg/kg in leaves against 800 mg/kg in fruits.

The average concentration of lithium in fruits and leaves was 9.59 and 41.68 mg/kg respectively. Lithium is another element with beneficial pharmacological properties; it has been used effectively in the treatment of manic depressive disorders. There is evidence to suggest that lithium is also an essential element [47].

The essential role of selenium (Se) for human health has been well established in recent years [44, 48]. Selenium ranges from 12.29 to 17.42 mg/ kg in *P.lentiscus* fruits and leaves respectively. Leaves contain highest concentration of Se, whereas fruits contain lowest concentration of Se.

The concentrations of manganese (Mn) vary significantly with the part of *P.lentiscus*. It ranged between 226.49 mg/kg in leaves and 171.28 mg/kg in fruits.

Cadmium and lead are best known for their toxicological properties [47]. There are increased in depressives and schizophrenics but reduced in manic patients [49].The concentrations of Cadmium are 3.96 mg/kg - 6.45 mg/kg in fruits and leaves respectively. Lead content are 11.5-25.81 mg/kg in fruits and leaves respectively. The higher concentrations of Cd and Pb in samples collected from traffic area may be due to automobile exhausts. Usually, automobile exhaust and smelters are the main sources of Pb and Cd respectively [50].

The difference in trace element concentrations between *P.lentiscus* leaves and fruits may be due to the metabolic function of each tissue [51].

3.3. Antioxidant properties

The antioxidant activity of the essential oil of leaves and fruits of *P. lentiscus* was determined by the DPPH test system. Table 3 demonstrates DPPH scavenging activity, expressed in percentage, caused by different concentrations of the essential oil of *P. lentiscus* (leaves and fruits).

Table 3: Percentage of DPPH radical scavenging as a function of concentration for essential oil from *P.lentiscus*

Sample	Antioxidant activities					
Essential oil of leaves (Saidia)	Essential oil concentration (µl/ml)	2	4	6	8	10
	% DPPH radical scavenging	24.48	28.84	32.42	33.05	33.21
	IC_{50} (µg/ml)	23.79				
Essential oil of leaves(Taforalt)	Essential oil concentration (µl/ml)	2	4	6	8	10
	% DPPH radical scavenging	21.12	21.26	21.54	23.98	52.11
	IC_{50} (µg/ml)	12.8				
Essential oil of fruits (Saidia)	Essential oil concentration (µl/ml)	2	4	6	8	10
	% DPPH radical scavenging	18.98	20.6	20..9	23.98	28.78
	IC_{50} (µg/ml)	27.65				
Ascorbic acid	Solution concentration (µg/ml)	2	4	6	8	10
	% DPPH radical scavenging	37.19	46.45	48.22	48.74	58.86
	IC_{50} (µg/ml)	6.69				

There is no significant difference between leaves and fruits essential oils antioxidant activity. The essential oils of both plant parts had low activity than natural antioxidant activity. But the leaves essential oil of Taforalt has a stronger activity than the essential oil of fruits and leaves of Saidia.

Therefore, DPPH scavenging activity is usually presented by the IC_{50} value. Concentration of the antioxidant providing 50% inhibition of DPPH in the test solution (IC_{50}) were calculated and presented in Table 3.

The activity of the essential oil is proportional to the concentrations and the lower IC50 value reflects better protective action. The leaves essential oil of Taforalt is able to reduce the stable free radical 2,2 0-diphenyl-1-picrylhydrazyl (DPPH) with an IC_{50} of 12,8 µg/ml.

The lowest IC_{50} value (highest antioxidant activity) of 12.8 µg/ml was obtained for leaves oil of *P. lentiscus* L. harvested of Taforalt, This value is found to be comparable to those found in *P. lentiscus* extracts (IC_{50} = 11 mg/l) [21].

The leaves and fruits essential oils of Saidia were found to be less efficient in radical scavenging with an IC_{50} value of 23.79 and 27.65µg/ml respectively, whereas this of the natural antioxidant activity is 6.69µg/ml.

The essential oil of the leaves of Taforalt had the highest radical scavenging activity with the lowest IC_{50} value of 12.8 µg/ml. this was higher than the essential oil of the leaves and fruits of Saidia with IC_{50} value of 23.79 µg/ml and 27.65µg/ml respectively. In addition, DPPH scavenging abilities of the essential oil of the leaves (Taforalt and Saidia) were less than that of the standard (ascorbic acid with an IC_{50} value of 6.69µg/ml).

Pistacia lentiscus essential oils contained monoterpene hydrocarbons and oxygenated monoterpenes such as α-pinene, β-pinene, 3-carene and β-myrcene. Moreover, trying to correlate the observed activity with the chemical composition of the oils, it is noteworthy to cite the work of Ruberto and Baratta[52], who studied the antioxidant activity of 98 pure essential oils chemical components and showed that monoterpene hydrocarbons had a significant protective effect, with several variants due to the different functional groups. This finding is in agreement with this work, Table 3 shows that leaves essential oil of *P. lentiscus* L. and the leaves essential oil of *P.lentiscus* of Taforalt are markedly rich in monoterpene hydrocarbons [53]. Furthermore, some researchers show

that some essential oils rich in nonphenolic compounds also have antioxidant potentials [54]. Table 1 show that essential oils of *P. lentiscus* are markedly rich in nonphenolic components. Antioxidant activities of *P. lentiscus* L. essential oils can be attributed to the nonphenolic constituents.

Conclusion

This study was conducted to investigate the essential oils of *P. lentiscus* from Eastern Morocco and in vitro evaluation of its antioxidant activities.

Significant qualitative differences were found in terms of chemical composition in the oils obtained from the investigated *Pistacia* parts (leaves and fruits).The fruits oil obtained from *P. lentiscus* was characterized by abundance of the oxygenated monoterpenes.

The results indicated that *P.lentiscus* L. leaves oil has an antioxidant activity against DPPH, and further support the view that *P. lentiscus* L. is promising sources of natural antioxidants.

The work on trace elemental analysis of various parts of *P. lentiscus* from Eastern Morocco using ICP-AES technique, exhibits two different elemental distribution patterns within the plant. Fruits contain the highest concentration of K and Ca, while the concentration of Mg, Cu, Fe, Mn, Li, Zn, Cr, V and La is the highest in leaves.

Acknowledgments: This work was supported by the University Commission for Development (CUD) of Belgium.

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