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Phenolic profile and effect of growing area on *Pistacia lentiscus* seed oil

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ABSTRACT

In this investigation, we aimed to study, for the first time, the phenolic composition of *Pistacia lentiscus* seed oils from different growing areas. Extraction of the phenolic fraction from oils was done by methanol/water. Phenolic profiles were determined using chromatographic analysis by High Performance Liquid Chromatography (HPLC-DAD/MSD) and its quantification was done using an internal standard which is unidentified in the studied oil (syringic acid). Forty phenolic compounds were quantified and only eighteen of them were identified. The eight studied oils showed different phenolic profiles. The total phenols amount varied from 538.03 mg/kg oil in Jbel Masour oils to 4260.57 mg/kg oil in oils from Kef Erraai. The highest amount of secoiridoids was reached by Bouchoucha oil containing 366.71 mg/kg oil of Oleuropein aglycon. Oils from Kef Erraai locality contained the highest concentrations in flavonols (377.44 mg/kg oil) and in phenolic acids (2762.67 mg/kg oil).

1. Introduction

Phenols are chemical compounds present naturally in many fruits and vegetables, especially in those containing a large amount of salicylates. Phenolic compounds give fresh and processed plant products some of their major organoleptic properties. In addition to their contributions to color and aroma, they play a determining role in terms of taste, especially the sensations of astringency and bitterness (Shahidi, 2000). Research into the benefits of polyphenols is gaining momentum, including the recognition that polyphenols play an important role in the prevention of degenerative diseases such as cancer, cardiovascular disease or osteoporosis (Halliwell & Gutteridge, 1999; Steinberg & Lewis, 1997). Indeed, thanks to their antioxidant and anti-inflammatory properties, these molecules can help limit the damage associated with aging. Phenols can prevent oxidative damages caused by reactive oxygen and nitrogen species, which are continuously produced in the human body. When there is an over-production of these species, a failure in the defense mechanisns and damage to valuable biomolecules (DNA, lipids, proteins) may occur. This damage has been associated with an increased risk of cardiovascular disease, cancer and other chronic diseases (Aruoma, 1998). Although abundant in our diet, the effects of these compounds on health depend on the amount consumed and their bioavailability that varies from one polyphenol to another (Ames, Shigenaga & Hagen, 1993; Halliwell, 2002).

Recently, the presence of phenolic compounds in seed oils has been discovered. Olive, corn and soybean oils were designated to be an important source of phenols. These compounds contribute to their oxidative stability and to their nutritional importance (Naz, Sheikh, Siddiqi & Sayeed, 2004). Several other edible oils, such as *Pistacia lentiscus* oil, were studied for its antioxidant, antimicrobial and anticancer properties (Mezni et al., 2014; Mezni, Aouadhi, Khouja, Khaldi & Maaroufi, 2015; Mezni et al., 2016). All these activities suggest that *P. lentiscus* seed oil may contain an important amount of phenols. However, to our knowledge, no studies were conducted on its phenolic composition. Only its fatty acids, tocopherols, carotenoids and sterols compositions were studied (Mezni et al., 2014; Trabelsi et al., 2012). Polyphenols can be used for food, cosmetic, or pharmaceutical purposes, and information on the concentration of these compounds in *P. lentiscus* seed oil is required, especially that its consumption and its use are in continuous increase. Knowledge of the phenolic profile of this oil could also diversify its uses and increase the industrial opportunities of exploitation for this product.

The objectives of the present study were to determine the phenolic profile of *P. lentiscus* seed oil and to evaluate the influence of the growing area on the polyphenol content.

This objective is in line with current needs to promote by-products, to evaluate better traditional products and to develop compositional databases to improve accuracy of antioxidants consumption data.

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Table 1
Geographic coordinates and bioclimatic characteristics of the sites of *Pistacia lentiscus* samples.

Site	Latitude (N)	Longitude (E)	Altitude (m)	Pm (mm)	Tm (°C)
Azib	37,209918	9,9767898	139	740.88	18.4
Oueslatia	35,853055	9,5919444	475	251.18	21
Bouchoucha	36,516285	10,4409765	220	358	17.9
Jbel Mansour	36,256876	9,7910438	378	1011.7	18.5
Kebouch	36,210616	8,8881085	709	1113.9	18.4
Kef Erraai	37,180010	9,3073743	113	740.88	18.4
Sidi Zid	36,746666	10,319166	676	358	17.9
Gouairia	36.7109535	8.6727619	567	1187.94	18.5

2. Material and methods

2.1. Plant material

Mature fruits of *Pistacia lentiscus* L. (lentisk-mastic tree) were harvested from eight localities of the North and Center of Tunisia (Table 1). Fruits were harvested from at least five trees in each locality.

The certified specimen (VS1-PL2009) was deposited at the Herbarium of the National Research Institute of Rural engineering, water and forestry-Tunisia.

2.2. Oil extraction

After cleaning, the fruits were ground using a metal grinder. The paste was then mixed, in a steam bath, for a half hour allowing small oil droplets to combine into bigger ones. The separation of the oil from the rest of the lentisk components was done with a hydraulic press. The average yield calculated relative to the fresh weight was estimated at 12%.

2.3. Extraction of the phenolic fraction

The method described by the International Olive Council (2007) was conducted for extraction of the phenolic fraction from *P. lentiscus* oils. 2 g of oil were weighed in a test tube with a screw cap and 50 µL of Syringic acid used as standard solution (at a concentration of 0.015 mg/mL) were added. The whole was mixed with 5 ml of methanol/water (80/20; v/v) for 1 min. The mixture was then isolated in an ultrasonic bath (at 50 W) for 15 min at room temperature and centrifuged at 5000 rpm for 25 min. The methanolic phase was removed and stored for later uses.

2.4. Chromatographic analysis of phenols by HPLC-DAD/MSD

High-performance liquid chromatography (HPLC) analyses were performed using a HP 1100 Series instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with a diode array UV–Vis detector (DAD). The separation was carried out by means of a specific column C18: LiChrospher100 (250 × 4 mm, 5 µm) at room temperature. The mobile phase consists of acetonitrile (solvent A) and water with 0.2% sulfuric acid (solvent B). The program gradient was: 15% A/85% B 0–12 min, 40% A/% 60B 12–14 min, 60% A/40% B 14–18 min, 80% A/20% B 18–20 min, 90% A/10% B 20–24 min, 100% A 24–28 min. The volume of the injection was 20 µL and the wavelengths were set at 280 nm for phenolic acids, phenyl ethyl alcohols and secoiridoids. The peaks were identified by the retention times compared to those of the pure controls (purity ≥98.0%, Sigma-Aldrich, France).

The used standards are: Gallic acid, Sinapic acid, Tyrosol, 3–4 dihydrobenzoic acid, 4-hydroxyphenylacetic acid, chlorogenic acid, Vanillic acid, *p*-coumaric acid, Ferulic acid, Trans-4-hydroxy-3-methoxycinnamic acid, *o*-coumaric acid, Oleuropein aglycon, Luteolin, Kaempferol, Naphtoresorcinol, Salycilic acid, Pinoresinol, trans-cinnamic acid, Apigenin, Coumarin, Carnosic acid, Trans cinnamic acid and Rosmarinic acid.

Quantification of these identified compounds was done using an internal standard which is unidentified in plant extracts (syringic acid). The Calculation of response factors of external standards (RF) was used for the validation of the analytical method according to the following formula:

$$RF = \text{area syringic acid/mg syringic acid injected}$$

All solvents were HPLC grade and filtered through a 0.45 µm nylon filter disk (Lida Manufacturing Corp., Kenosha, WI, USA) prior to use.

2.5. Statistical analysis

The Data reported in this study were analyzed using the SAS 9.0 (Statistical Analysis System-GLM procedure) statistical software. The significance of differences at a 5% level between averages was determined by one-way ANOVA. The values of different parameters were expressed as the mean of three replicates ± standard deviation.

Principal component analysis (PCA) was conducted using R 3.1.1 software.

3. Results and discussion

For the different studied oils, an average of forty phenolic compounds was quantified and only eighteen of them were identified: Gallic acid, Tyrosol, 4-hydroxyphenylacetic acid, Vanillic acid, *p*-coumaric acid, Ferulic acid, Trans-4-hydroxy-3-methoxycinnamic acid, *o*-coumaric acid, Oleuropein aglycon, Luteolin, Kaempferol, Naphtoresorcinol, Salycilic acid, Pinoresinol, Apigenin, Coumarin, Carnosic acid and Trans cinnamic acid (Table 2).

There were 18 compounds found in Kebouch oil; 17 in Gouairia, Jbel Mansour, Kef Erraai and Oueslatia oils; 16 in Azib oil, 15 in Sidi Zid and 12 in Bouchoucha oil.

The eight studied oils showed different phenolic profiles (Fig. 1). The total phenols amount varied from 538.03 mg/kg oil in Jbel Masour oils to 4260.57 mg/kg oil in oils from Kef Erraai.

It is well known that the amount of phenolic compounds is an important factor when evaluating the quality of edible oils because of their contribution in its resistance to oxidation (Morello, Motilva, Tovar & Romero, 2004). *Pistacia lentiscus* seed oils showed a high amount of phenolic acids when compared with other edible oils such as olive oil and coconut oil (Krichene et al., 2007; Marina, Che Man, Nazimah & Amin, 2009). Several studies have reported that phenols are associated with dietary and sensory quality of oils. While at high concentrations they might contribute to dark color, astringent taste and off-flavor of some oils (Shahidi, 2000). This could explain the strong odor and pungent taste that characterizes *P. lentiscus* oils. Phenols have been reported, by several studied, to be an important antioxidants and to prevent oxidative damage to several molecules. Their role in chronic diseases treatment (such as cancer and cardiovascular disease) was also highlighted (Hallman, 2001; Visioli & Galli, 1998).

Oils from Kef Erraai locality contained the highest concentrations in flavonols (377.44 mg/kg oil) and in phenolic acids (2762.67 mg/kg oil). Vanillic acid was the dominant compound in this oil with 980.75 mg/kg oil. The highest amount of secoiridoids was reached by Bouchoucha oil containing 366.71 mg/kg oil of Oleuropein aglycon. Oil from Kebouch locality showed the most important amount of flavones (1305.12 mg/kg oil).

Phenolic profile of this oil showed that it is mainly composed of phenolic acids and flavones. These two compounds were considered as therapeutic agents. It was reported that phenolic acids and flavones have anti-carcinogenic and anti-mutagenic effects and that they are considered as neuroprotective agents in some neurodegenerative disorders such as Parkinson's and Alzheimer's diseases (Amyloid, 2002; Dai, Borenstein & Wu, 2006). Mennen, Walker, Bennetau-Pelissero and