Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):1-8

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phenolic Compounds Characterization from *Pistacia lentiscus* (lentisc) Fruit

Hajer Trabelsi[1*], Houda Ben Lajnef[2], Kawther Ben Arfa[1] and Sadok Boukhchina[1]

[1]*Université de Tunis El Manar, Faculté des Sciences de Tunis, Unité de Biochimie des Lipides, 2092 El Manar II, Tunisie.*
[2]*Université de Tunis El Manar, Faculté des Sciences de Tunis, Unité de Molécules Bioactives et Réponse Cellulaires au Stress Environnementaux, 2092 El Manar II, Tunisie.*

ABSTRACT

The total phenolic (TPC) content and flavonoids from fruits of three populations of Pistacia lentiscus (lentisc) were measured. Additionally, the polyphenol profile was analyzed using ESI-QTOF, MS/MS. The colorimetric assays revealed high levels in flavonoïds (from 13.78 mg RE / g dw (RM) to 23.46 mg RE / g dw (TB)) and in total phenolic compounds (from 24.84 mg GAE / g dw (KO) to 46.07 mg GAE / g dw). Furthermore, the main polyphenols are p-hydroxybenzoic acid, gallic acid, cinnamylidene acetic acid, quinic acid, p-coumaric acid 4-O-glucoside, 5-galloylquinic acid, isomer of caffeoylquinic acid, 3,4,5 O-trigalloylquinic acid, quercetin, taxifolin, quercetin-3-O-glucuronide, luteolin 6,8-di-C-hexoside, oleoside and epirosmanol. From a nutritional standpoint, these results show that lentisc fruits have a promising food value.

Keywords: Phenolic compounds, flavonoids, ESI-QTOF, MS/MS, spectrophotometric assay.

INTRODUCTION

Pistacia lentiscus is a particularly representative forest shrub of the hottest areas of the Mediterranean climate, characterized from the ecological point of view, for a high tolerance to climatic variations. It can grow under relatively low rainfall slices and adapts to all soils . In Tunisia, this species is common in the northern region and becomes more scarce in the center and south. Traditionally, in the Tunisian population, the fruit of *Pistacia lentiscus* is known for its many therapeutic effects, it is appreciated in the treatment of scabies, rheumatism, minor burns and in the manufacture anti-diarrheal medicine [1]. It is also taken orally to treat respiratory allergies and the stomach ulcers [2]. Its oils are monounsaturated and their high content of essential fatty acids demonstrates the importance of their food value [3-4].

In addition to traditional primary metabolites: lipids, carbohydrates, amino acids and proteins, plants often accumulate a diverse group of secondary metabolites that play a major role in the digestibility in the physiological protein utilization and in the plant defense against environmental stress [5]. Eighty percent of the phenolic compounds are mainly in the epidermal tissue of the fruits. These are phytomicronutriments and pigments generally responsible for autumnal colors of leaves and flowers (yellow, orange, red) [6]. Phenolic compounds are biologically active molecules having one or more benzene ring bearing one or more hydroxyl functions [7]. These compounds consist of approximately 8000 different molecular species and are divided into several categories: phenolic acids, flavonoids, tannins obtained by polymerization of flavonoids, lignans and coumarins. Polyphenols are the most abundant antioxidants in our diet derived from intakes of fruits, vegetables and grains. They are also used as additives in certain food products, pharmaceutical and cosmetic industries [8]. These compounds can protect the cell against oxidation and reduce the risk of various degenerative diseases associated with oxidative stress components. They are also involved in the prevention of the body against cardiovascular disease, cancer, diabetes and neurodegenerative diseases [9]. Different methods of analysis of phenolic compounds are available. Most

common in the literature is high-performance liquid chromatography (HPLC), but to improve the result, HPLC can be coupled with other techniques such as mass spectrometry [10]. This coupling allows a better identification of the eluted compounds but it has still many constraints hindering its full implementation.

Many studies have determined the phenolic composition of *Pistacia lentiscus* resin and leaves, but no studies have yet been conducted to determine the phenolic composition of these fruits. The aim of this work was to identify, for the first time, the phenolic compounds present in the methanolic extracts from *Pistacia lentiscus* fruits, using the QTOF-ESI-MS/MS and to determine their contents in total phenolic compounds and in flavonoids, based on spectrophotometric assays.

EXPERIMENTAL SECTION

Reagent and standards

Methanol, acetic acid, catechin, vanillin, rutin, gallic acid and Folin-Ciocalteu reagent were acquired from Sigma Aldrich (Paris, France). NaOH was purchased from Merck (Darmstadt, Germany) and Na_2CO_3, $AlCl_3$, $NaNO_2$, H_2SO_4 were obtained from Fisher Scientific SA (Loughborough, Spain).

Samples

P. lentiscus fruits of L. (Lentisc), at full maturity, being in the wild, were taken from three regions of Tunisia: Korbous (KO) (Northeast of Tunisia, at 36 ° 49'N of latitude and 10 ° 34 'E of longitude, characterized by a subhumid climate and a sandy clay soil), Tebaba (TB) (Northwest of Tunisia, at a latitude of 37 ° 00'N and a longitude of 9 ° 06 'E, characterized by a humid climate and a sandy clay soil) and Rimel (RM) (North of Tunisia, at 37° 14'N of latitude and 9° 54' E of longitude, characterized by a subhumid climate and a sand dune limestone soil) in January 2009. The fruits from each region were collected from 10 trees and mixed for further analysis.

Spectrophotometric determination of antioxidant

Methanol extract preparation

A sufficient amount (7 g) of the fruit of *Pistacia lentiscus* was finely ground in a mortar and was stirred with 70 mL of methanol at 30 °C for 24 hours. The extract was filtered through No.1 Whatman filter paper. The residue was re-extracted with 70 mL of methanol. The extracts were filtered again, combined and concentrated under vacuum at 40 °C, and they subsequently used for spectrophotometric assays of flavonoids, condensed tannins and total phenolic compounds.

Determination of total phenolic compounds

Total phenolic compounds content was determined using the Folin-Ciocalteu reagent [11]. The latter is constituted by a mixture of phosphotungstic and phosphomolybdic acids which is reduced during the phenols oxidation in mixture of the blue oxides of tungsten and molybdenum. At a well-defined volume of the previously prepared methanolic extract (0.125 mL) was added 0.5 mL of distilled water, 0.125 mL of Folin-Ciocalteu, and 1.25 mL of sodium carbonate (Na_2CO_3, 7%) to promote an alkali environment and initiate the redox reaction. The mixture is then incubated in the dark for 2 hours. The absorbance is measured at 760 nm using an UV spectrophotometer. A reference range based on gallic acid is prepared in parallel and under the same conditions. The total phenolic compound contents are in milligram of gallic acid equivalent per gram of dry matter (mg GAE / g DW).

Determination of flavonoids content

The method used is based on the complex formation between the aluminum chloride (AlCl3) and flavonoids. The orange color intensity indicates the importance of flavonoid content in plants.

Seventy-five microliters (75 µL) of $NaNO_2$ (5%) was added to 250 µL of the methanol extract. After six minutes, a freshly prepared $AlCl_3$ solution are added to 150µL. A second incubation for 5 min at room temperature is performed, followed by the addition of 0.5 mL of NaOH (1M). The mixture was then adjusted with distilled water to a final volume of 2500µL. The absorbance reading was performed at 510 nm [11]. A calibration curve based on rutin at different concentrations was prepared in parallel and under the same conditions of flavonoids quantification. The results are expressed in mg equivalent of rutin per gram of dry Weight (mg QE/g DW).

Identification of different phenolic molecules using ESI-QTOF MS, MS / MS

Extraction of phenolic compounds

Ten milliliters of pure methanol were added to an appropriate amount (1g) of the dehydrated and powdered plant material. After intense agitation by vortex for 30 min, the mixture is incubated in the dark for 24 h at 4 °C, then filtered through a Whatman paper n.4 and evaporated to dryness using a rotary evaporator. The residue was

redissolved in methanol/ water (1: 2 v/v) and eluted over a solid-phase extraction C-18 column (SPE) of Supelco Type.

The solid phase extraction was carried out in four successive stages:
- The SPE column was activated by percolating 1 mL of methanol followed by 1 ml of methanol/water (1: 1 v/v) using a Visiprep SPE Vacuum Manifold (Sigma Aldrich, Alberta, Canada). The column must not dry before the loading of the sample.

- The sample was loaded onto the SPE cartridge dropwise

- The column was washed with 1 ml of acidified water to remove the sugars and other polar compounds.

- Phenolic compounds were eluted with 2 mL of 90 % methanol.

The residue obtained was filtered through a microfilter of 0.45 µm and was used for the subsequent identification of various phenolic compounds.

Analysis of phenolic compounds from the fruit of *Pistacia lentiscus* using the ESI-QTOF-MS, MS / MS
Various phenolic compounds are detected using a quadrupole time of flight (QTOF) mass spectrometer (Waters), with electrospray ionization (ESI), operating in the negative ionization mode and using control software Mass Lynx (ver. 4.0). Twenty microliters of the residue obtained were injected directly into the loop, and carried by the mobile phase (composed of methanol and water with 0.5% acetic acid) with a flow of 150 µL / min. The source temperature was 100 °C and the cone voltage was set at 50 V. The drying gas N_2 has a flow rate of about 540 L / h.

The identification of the compounds was carried out by referring to the literature [12-13-14] and was confirmed by MS / MS.

The collision energy was optimized for each compound varied from 15 to 40 eV. LM and HM quadrupole resolution was varied between 12 and 16 and the argon (target gas) was held at a constant pressure in the collision cell. Most of the MS/MS spectra of the phenolic compounds were obtained automatically using a data dependent survey scan. The MS analysis was performed between a mass range of 50 to 1100 m/z. When a signal from the mass spectrum of sufficient intensity is reached, the peak selected automatically. The test takes 20 seconds. Thereafter, the instrument returns to the MS mode until a subsequent signal of sufficient intensity is reached and the process repeats. Unselected phenolic compounds of interset were analyzed later by manually selection of these peaks.

Statistical analysis
The experimental data were analyzed using a Statistical Analysis System (XLSTAT 2014). Results are presented as mean and standard deviation (SD). Differences were considered statistically significant by Duncan's new multiple range test at $P \leq 0.05$.

RESULTS AND DISCUSSION

Total phenolic compounds of *Pistacia lentiscus* fruits
The total polyphenol content of the *Pistacia lentiscus* fruits extract is determined by the Folin-Ciocalteu assay at a wavelength of 760 nm. A calibration curve was established with the gallic acid and the results are expressed in mg GAE/g dry weight (DW).

Total phenol compounds (TPC) of fully ripened *P. lentiscus* fruit from different Tunisian populations varied from 24,84 mg GAE/g (KO) to 46,07 mg GAE/g (TB) on a dry weight basis (Table 1). Significant difference ($p<0.05$) between the study populations was recorded, which may be due essentially to geographical conditions, climate and soil. Comparing these results with literature, the content of TPC from the *Pistacia lentisus* fruit is high to that obtained in nuts such as hazelnuts (22.5 mg / 100g), cashews (86.7 mg / 100g) [15], Soja (0.9 mg GAE/g dw) and grenade (1.38 mg GAE/g dw) [16-17]. Also, our results are high compared with those obtained from a related species of the Anacardiaceae family *Pistacia vera* from Argentina and Turkey (between 360 and 463 mg GAE/g dw) [18-19]. On the other hand, our results are near to those obtained by [20] by studying the composition of the Iranian *Ocimum basilicum* (from 22.9 to 65.5 mg GAE/g of dry material). In addition, the TPC amount of *Anthemis arvensis* is similar to that obtained at the Rimel station [21].

The richness of *P. lentiscus* with these compounds confirms its nutritional and medicinal value and it can be used as a potential source of phenols. Indeed, their important role is widely shown in the protection against certain diseases because of their possible interaction with many enzymes and their antioxidant properties [22]

Table 1: Total polyphenols, flavonoids and condensed tanins compositions of *Pistacia lentiscus* fruit

Stations	Total phenolic content (mg GAE/g DW)	Flavonoids (mg RE/g DW)
Rimel (RM)	32,47±1.12[b]	13,78±1.44[b]
Korbous (KO)	24,84±1.79[c]	22,90±0.39[a]
Tebaba (TB)	46,07±2.74[a]	23,46±4.72[a]

Data followed by different letters are significantly different from other ($p<0.05$) according to Duncan's test.

Flavonoids of *Pistacia lentiscus* fruits

The flavonoid class of molecules includes many natural compounds divided into several families. The most important of which are catechins, quercetin and isoflavones. These are polyphenolic pigments which are responsible for most of the colorations in flowers and fruits [23]. There have been many therapeutic virtues and they are particularly active in maintaining good circulation. Some also have anti-inflammatory and anti viral properties, others have protective effects on the liver [24].

The dosage of flavonoids was performed according to the method described by Dewanto et al. [11], using rutin as standard. Inspection of Table 1 shows that the flavonoïd content varies from 13.78 mg RE/g dw (RM) to 23.46 mg RE/g dw (TB). This significant difference between the two populations of Rimel and Tebaba could be due to geographical conditions, climate and soil.

Our results show that *Pistacia lentiscus* is rich in flavonoïds in comparison with other herbs (*Ruta montana*: 1.62 mg RE / g dw), (*Thymelaea hirsuta*: 4.95 mg RE / g dw), *Oudneya africana*: 7.66 mg RE / g dw) and (*Artemisia herba halba*: 11.31 mg RE / g dw) [21].

The high content of flavonoids detected in our samples is interesting, this gives them an important nutritional and therapeutic value. Indeed, these compounds are capable of inhibiting carcinogenesis [25] and to protect the gastric mucosa against various ulcerogenic agents [26].

Identification of various phenolic compounds from *Pistacia lentiscus* fruit using ESI-QTOF-MS, MS/MS

Phenolic compounds of *Pistacia lentiscus* fruit were detected and identified using an electrospray ionization quadrupole time-of-flight mass spectrometer QTOF2 (Waters), operating in negative ion mode and using a checking and processing software Mass Lynx (v. 4.0). This technique allows detection of molecular species even at trace.

Figure 1 shows the ESI-QTOF-MS spectrum of the methanol extract of the *Pistacia lentiscus* fruit. Fourteen phenolic compounds were identified (Figure 2 and Table 2), which are: 8 phenolic acids (p-hydroxybenzoic acid, gallic acid, cinnamylidene acetic acid, quinic acid, p-coumaric acid 4 -O-glucoside, 5-galloylquinic acid, isomer of caffeoylquinic acid and 3,4,5 O- trigalloylquinic acid), 4 flavonoïds (quercetin, taxifolin, quercetin-3-O-glucuronide and luteolin 6,8-di-C-hexoside), one secoiridoïd (oleoside) and one phenolic diterpene (epirosmanol).

Analysis of the resin of *Pistacia lentiscus* by Kaliora *et al.* [27], using HPLC and GC-MS has allowed the identification of 15 different phenolic compounds which are cinnamic acid, tyrosol, p-hydroxybenzoic acid, p-hydroxyphenylacetic acid, vannilic acid, homovanillic acid, *O*-coumaric acid, protocatechuic acid, 3-4-dihydroxyphenylacetic acid, syringic acid, p-coumaric acid, gallic acid, ferulic acid, caffeic acid and sinapic acid.

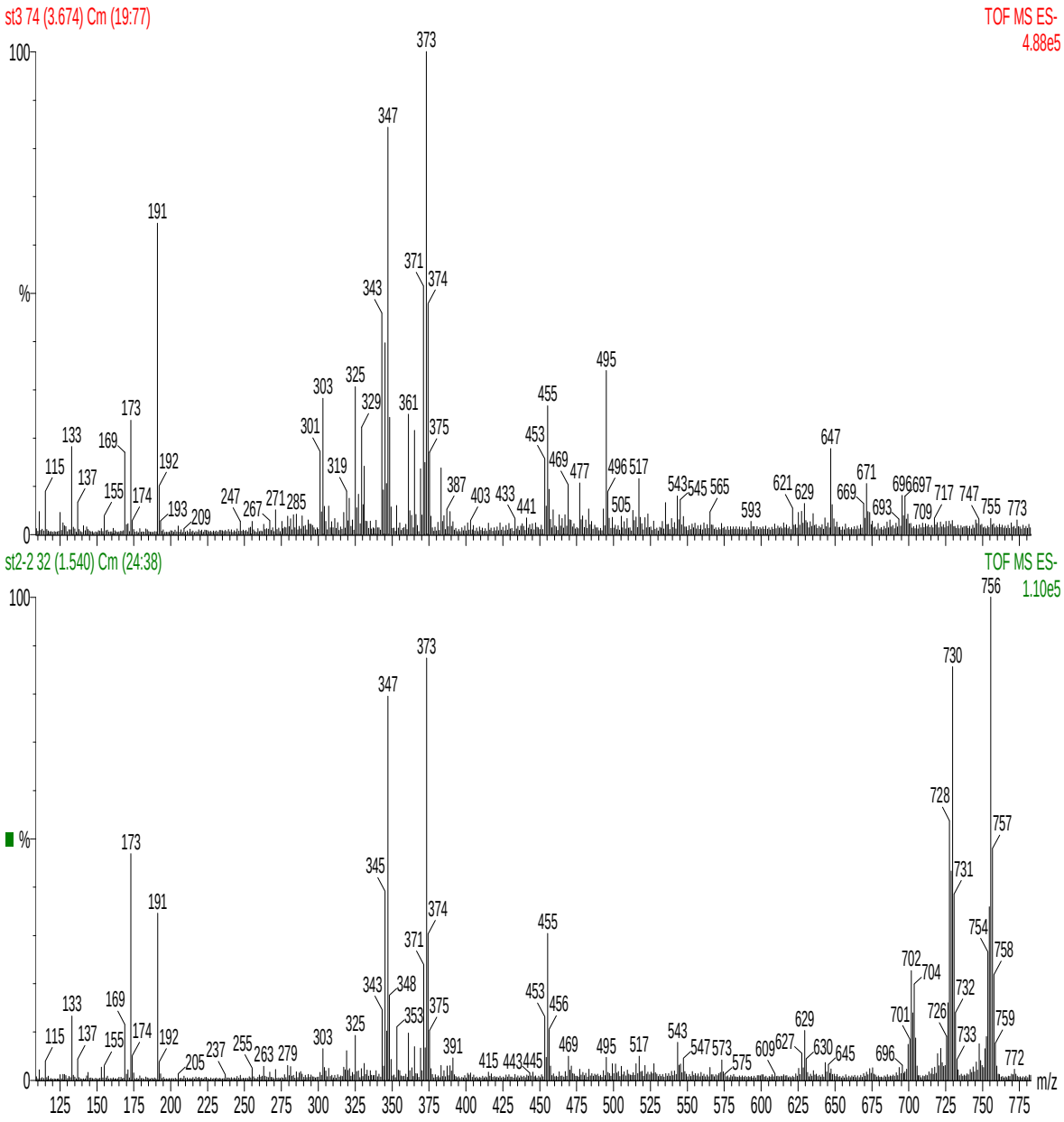

Figure 1: ESI-QTOF-MS spectra of the methanol extract of *Pistacia lentiscus* fruit

Table 2: ESI-QTOF-MS, MS/MS fragmentation of compounds identified in the methanol extract of the *Pistacia lentiscus* fruit

ESI-QTOF-MS		
Phenolic compounds identified	Ions $[M-H]^-$ *m/z*	Ions MS/MS *m/z*
p-hydroxbenzoïc acid	137	93
Gallic acid	169	125
Cinnamylidene acetic acid	173	115,129, 111, 137
Quinic acid	191	85, 93, 111, 127, 173
Quercetin	301	151
Taxifolin (dihydroquercetin)	303	285
p-coumaric 4-O-glucoside acid	325	---
5-Galloylquinic acid	343	169, 191
Epirosmanol	345	301
Cafeoylquinic acid isomer	353	111, 173, 191
Oleoside	389	345
Quercetin-3-O-glucuronide	477	179, 301
Luteolin 6,8-di-C-hexoside	609	399, 429
3,4,5,-O- Trigalloylquinic acid	647	495

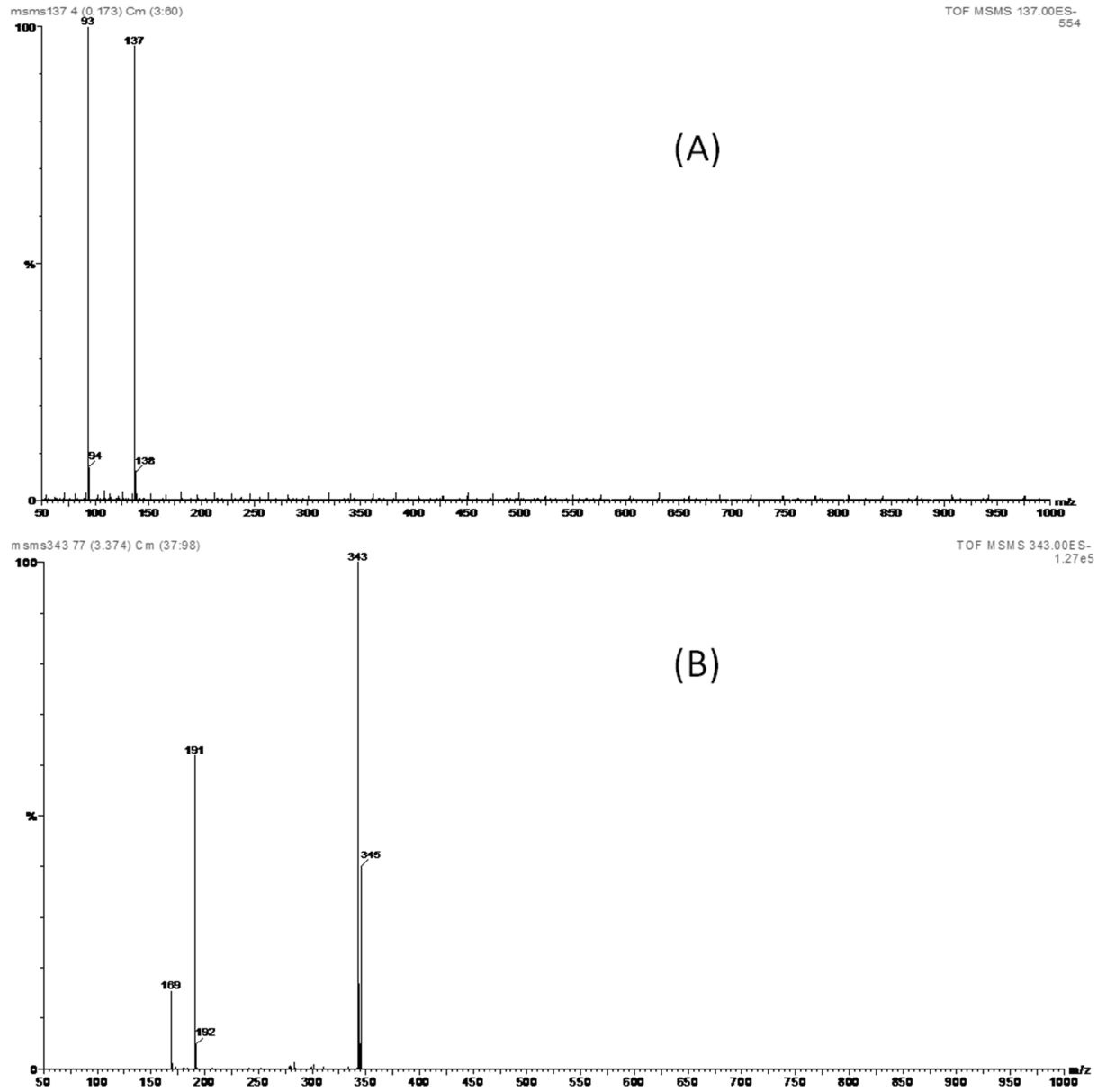

Figure 2: MS/MS spectra of some identified phenolic compounds. A: p-hydroxbenzoïc acid; B: 5-Galloylquinic acid

Generally, the phenolic compounds are involved in interactions of plants with their environment, by playing the role of certain pathogenic recognition signals or allowing them to withstand to various biotic and abiotic stresses. According Boutigny and et al. [28], phenolic acids significantly inhibit the accumulation of mycotoxins in wheat. Moreover, these compounds which represent the major portion of polyphenols identified in lentisc fruit, have physiological effects well expressed: anti-inflammatory, anti-bacterial, sedative, hepatoprotective [29]. Other studies have demonstrated the inhibitory effect of gallic acid, quinic acid and its derivatives galloyles on the oxidation of LDL lipoprotein [30]. Four different molecules belonging to the class of flavonoids have been identified in our samples. These phenolic compounds may exert a variety of biological activities including antioxidant, vasculoprotective, antihepatotoxic, antiallergic, anti-inflammatory, anti-ulcer and even significant antitumor properties. They are also able to modulate the activity of certain enzymes and to change the behavior of several cell systems [31]. Previous work has demonstrated the role of quercetin in inhibiting the growth of cancer cells [32] and in protection against cardiovascular disease [33]. Similarly, this molecule appears to have antiulcer effects [34], as well as antioxidant, antiallergic, antiviral and anti-inflammatory activities [35]. Furthermore, taxifolin is a powerful flavonoid with more therapeutic effects. In fact, it protects cell membranes, improves the activity of capillaries and blood microcirculation throughout the body and normalizes metabolism at the cellular level. It also has anti-inflammatory and hepatoprotective activities, a anti-edematous effect, it reduces cholesterol levels and reduces clotting and blood viscosity [36-37]. The oleoside (*m/z* 389) detected and identified in our samples, is a phenolic molecule belonging to the class of Secoiridoid. These molecules have been identified primarily in olives and olive

oil [38], they prevent or retard the growth rate of a range of bacteria and micro-fungi, but to our knowledge, there is no data in the literature about the possible use of these secoiridoid as antimicrobial agents against pathogenic bacteria in humans [39]. If we consider the direct food use of polyphenols, consumption of 10 to 100 mg of a given phenolic compound significantly increases the antioxidant capacity of plasma serum, this increase could reach 20% within 2 hours after consumption foods rich in polyphenols [40].

CONCLUSION

In the present study, the total phenolic and flavonoid contents from three populations of *Pistacia lentiscus* were evaluated spectrophotometrically. Moreover, the identification of its various phenolic compounds has been performed for the first time by ESI-QTOF MS / MS. The main results of our study are summarized as follows: the fruit of *Pistacia lentiscus* is characterized by a high content of total phenolics and flavonoids and by the presence of eight phenolic acids, four flavonoids, one secoiridoid and one phenolic diterpene.

Acknowledgments
We thank the Ministry of Higher Education and Scientific Research of Tunisia for financially supporting this investigation, the members of the Mass Spectrometry Laboratory (University of Ottawa, Ottawa, Canada) for providing constructive remarks and the INRGREF for help in collecting the samples, in particular, Mr Mohamed Larbi Khouja.

REFERENCES

[1] E Le Floc'h; M A Nabli. Ministère de l'enseignement supérieur et de la recherche scientifique, *Imprimerie officielle de la République Tunisienne*, **1983**, pp. 144-145.
[2] P Iserin. (2ème édition) Ed Larousse/VUEF, 2001, 13-16pp, 250p, 291-296pp.
[3] H Trabelsi; O A Cherif; F Sakouhi; P Villeneuve; J Renaud; N Barouh; S Boukhchina; P Mayer. *Food Chem*, **2012**, 131, 434-44.
[4] H Trabelsi; F Sakouhi; J Renaud; P Villeneuve; M L Khouja; P Mayer; S Boukhchina. *Eur J Lipid Sci Tech*, 2012, 114, 968-973.
[5] M Naczk; F Shahidi. *J Chromatography*, **2004**, 1054, 95-111.
[6] R Jussi-Pekka. Academic Dissertation, **2001**, Helsenki.
[7] L Bravo. *Nutrition Review*, **1998**, 56, 317-333.
[8] K Chira; J H Suh; C Saucier; P L Teissèdre. **2008**, 6, 75-82.
[9] A Scalbert; C Manach; C Morand; C Remesy; L Jimenez. *Crit Rev Food Sc Nutrition*, **2005**, 45, 287-306.
[10] V Carbon; P Montoro; N Tommasi; C Pizza. *J Pharmaceutical Biomed Anal*, **2004**, 34, 295-304.
[11] V Dewanto; X Wu; K K Adom; R H Liu. *J Agri Food Chem*, **2002**, 50, 3010-3014.
[12] D Jianpeng; S Y L Viola; T C T Jason, L Maw-Rong. *J Agri Food Chem*, 2007, 55 (18), 7462-7468.
[13] K Banaszewski; I Edirisinghe; B Burton-Freeman; J C Cappozzo. 58th ASMS conference on Mass Spectrometry and Applied Topics, **2010**, Salt Lake City, Utah.
[14] W Y Jeong; S J Jong; A C Yong; H L Jung; P Semin; W J Sung; Y M Kim; C S Lim; A M Abd El Aty; G S Kim; J L Soo; J H Shim; S C Shin. *J Separation Sc*, **2011**, 34 (21), 2967-2974.
[15] Yang J. *J Food Sci Tech*, **2009,** 42: 1573-1580.
[16] S Zlic, V Sukalovic, T Hadzi, V Maksimovic. *46th Croatian and 6th International Symposium on Agriculture*, **2005**, Opatija. Croatia. p. 689.
[17] J Pu; Y Tian; S Haiming; S Yi; S Margaret; G Boyan; L Linwei; Y Liangli. **2012**, 132, 1457-1464.
[18] I Arcan; A Yemenicioglu. *J Food Comp Anal*, **2009**, 22, 184–188.
[19] M P Fabani; L Luna; M V Baroni; V Magdalena; M I Monferran; A Tapia; DA Wunderlin; G E Feresin. *J Functional Foods*, **2013**, 5, 1347-1356.
[20] J Javanmardi; C Stushnoff; E Locke; J M Vivanco. *Food Chem*, **2003**, 83, 47-550.
[21] A Djeridane; M Yousfi; B Nadjemi; D Boutassouna; P Stocker; Vidal N. *Food Chem*, **2006**, 97, 654-660.
[22] A Fleuriet; C Jay-Allemand; J J Macheix. *Presses polytechniques et universitaires romandes*, **2005**,121-216pp.
[23] L Peng; W Bochu; P Ren. Colloids surfaces B, *Biointerfaces*, **2005**, 45, 108-111.
[24] A E Kirk, P Sutherland; S A Wang; A Chait; R C LeBoeuf. *J Nutrition*, **1998**, 128 (6), 954-959.
[25] C A Rice-Evans, N J Miller. *Biochem Soc Trans*, **1996**, 24, 790-795.
[26] A Villar, M A Gasco, M J Alcaraz. *J Pharmacy Pharmacology*, **1987**, 39, 502-7.
[27] A C Kaliora; A Mylona; A D Chiou; G N Petsios; K Andrikopoulos. *J Liquid Chrom Related Technol*, **2004**, 27, 289–300.
[28] A L Boutigny; F Richard-Forget; C Barreau. *Eur J Plant Pathology*, **2008**, 121 (4), 411-423.
[29] L A Garcia; A J Vélez; A P De Rozo. *Archivos Latinoamericanos de Nutricion*, **1985**, 35 (3), 491-495.
[30] MC Baratto; M Tattini; C Galardi; P Pinelli; A Romani; V Visioli; B Basosi; R Pogni. Free *Radical Res*, **2003**, 37, 405-412.

[31] K Ghedira. *Phytothérapie*, **2005**, 3 (4), 162:169.
[32] M A Avila; J A Velasco; J Cansado; V Notario. *Cancer Res*, **1994**, 54, 2424- 2428.
[33] P S Brookes; S B Digerness; D A Parks; V D Usmar. *Free Radical Biol Med*, **2002**, 32(11), 1220-1228.
[34] Tan PV, Nditafon NG, Yewah MP, Dimo T, Ayafor FG. **1996**. Eremomastax speciosa: effects of leaf aqueous extract on ulcer formation and gastric secretion in rats. J Ethnopharmacology 54: 139-142.
[35] M T Gatto; S Falcocchio; E Grippa; G Mazzanti; L Battinelli; D Lambusta; L Saso. *Bioorganic Med Chem*, **2002**, 10, 269-272.
[36] Y O Teselkin, I V Babenkova, V K Kolhir, A I Baginskaya, N A Tjukavkina, Y A Kolesnik, I A Selivanova, A A Eichholz. *Phytotherapy Res*, **2000**, 14, 160-162.
[37] H Dok-Go; K H Lee; H J Kim; E H Lee; J Lee; Y S Song; Y Lee; C Jin; Y S Lee; J Cho. *Brain Res*, **2003**, 965, 130-136.
[38] C Soler-Rivas; JC Espin; HJ Wichers. *J Sci Food Agri*, **2000**, 80, 1013–1023.
[39] G Bisignano; A Tomaino; R Lo Cascio; G Crisafi; N Uccella; A Saija. *J Pharmacy Pharmacology*, **1999**, 51 (8), 971-974.
[40] J J Macheix, A Fleuriet; C Jay-Allemand. *Presse polytechniques et universitaires Romandes*, **2005**, p148.