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Phytochemical Composition and Antioxidant Activity of *Pistacia lentiscus* L. Leaves and Berries Oilcake Extracts

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ABSTRACT

As a part of prospecting bioactive molecules from natural resources, the phytochemical composition and antioxidant properties of extracts of leaves and berries oilcake of *Pistacia lentiscus* L., collected from two sites (Mechat and Bordj-Ali) in the northeast region of Algeria, were investigated. Dried leaves and berries oilcake obtained after removing the oily part by mean of Soxhlet apparatus were macerated in methanol in order to recover their respective active extracts. The phytochemical content analysis showed that lentisk leaves of both sites were relatively rich in polyphenols, flavonoids, flavonols and proanthocyanidins. The phytochemical content of berries oilcake extract was lower than that of leaves, yet, samples of Bordj-Ali displayed higher values for all assayed phytochemicals compared to those of Mechat. The *P. lentiscus* leaves extract exhibited a strong radical-scavenging activity (IC_{50} = 10.46 µg/mL) against 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals and a substantial inhibitory activity against H_2O_2 free radicals (20.23~25.92%). Furthermore, these extracts displayed a very strong reducing power (EC_{50} = 28.08 µg/mL) and total antioxidant capacity (104.07~159.39 mg EAA/g DW). The observed results correlated positively with total phytochemical content strongly plead in favor of valorization of this wild plant as a potential natural source of active biomolecules for food, cosmetics, and medicinal industry sectors.

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Introduction

Pistacia lentiscus L., commonly known as mastic or lentisk tree, is a species that belongs to the Anacardiaceae family. It extends east from the Asian site, Europe and Africa to the Canary Islands west (Bellakhdar, 2003). It is a very common shrub in the Mediterranean area. In Algeria, the lentisk tree grows spontaneously in sub-humid and semi-arid regions (Smail-Saadoun, 2005), especially in the forest and Tellian areas all along the northeast coast of the country. The lentisk tree is identified by its leaves alternate with a winged petiole and paripinnate, arranged in two rows, composed of 2-3 pairs of elliptical-obtuse leaflets. Its fruiting starts in autumn with the appearance of small globular fruits; a red drupe, then black when ripe; about five millimeters which contains a single single-seeded nucleus (Ain-lhout et al., 2004).

Lentisk tree is considered a multipurpose species, especially for its resin and oil, as well as for the benefits of its leaves and branches. This plant is known since ancient times for its medicinal properties (Dimas et al., 2009; Rodríguez-Pérez et al., 2013; Bampouli et al., 2014; Foddai et al., 2015; Pachi et al., 2020).

The vegetable oil obtained after extracting the fruits finds its use in various treatments; scabies, rheumatism, stomach, and respiratory infections, wound healing. Even the waste from crushing, or cake, is used in the feed of livestock and poultry (de Lanfranchi et al., 1999).

Usually, the aerial parts; leaves, twigs, and flowers, have an antifungal effect (Mansour-Djaalab et al., 2012). The infusions of the leaves help in the treatment of eczema, diarrhea, and throat infections (Rodríguez-Pérez et al., 2013). Some studies have clearly shown that the leaves of the lentisk tree exhibit several biological activities through their antibacterial (Mharti et al., 2011), anthelmintic (Landau et al., 2010; Manolaraki et al., 2010; Azaizeh et al., 2013), and Anticoccidian effect (Markovics et al., 2012).

In addition to its therapeutic effects, anthocyanins from *P. lentiscus* berries are used in the food industry as a food colorant (Longo et al., 2007).

Recently, a survey conducted on the use of *P. lentiscus* in three areas of northern Algeria revealed that the most treated diseases were respiratory, digestive, and circulatory disorders and allergies (Boudieb et al., 2019).

Previous works have addressed several aspects that lentisk derivatives can deal with. Ammari et al., (2018) investigated the neuroprotective and hepatoprotective activity of *P. lentiscus* oil; they suggested that it may serve as an anti-amnesic. Balan et al., (2007) evaluated the inhibition of human colon cancer cell proliferation by mastic gum extract. They suggested developing it into a chemotherapeutic agent. Cheurfa and Allem (2015) reported the hypocholesterolemic properties of *P. lentiscus* leaves extract. Dellai et al., (2013) investigated the anti-inflammatory and anti-ulcerogenic activities of *P. Lentiscus* leaves aqueous and organic extracts in rats. They observed an anti-inflammatory effect and an inhibition of gastric lesions in a dose-dependent manner. Furthermore, Foddai et al., (2015) considered the antiobesity-diabetes therapeutics from Sardinian *Pistacia* sp. They found that fruit and leaves water-soluble phytochemicals can inhibit crucial gastrointestinal enzymes involved in carbohydrate and lipid digestion and absorption. According to Remila et al., (2015) *P. lentiscus* berries and leaves crud extracts exhibited a strong antioxidant and significant cytoprotective activity. They reported that they inhibited the growth of melanoma B16F10 cell lines.

The investigations on the chemical composition and biological activity of *P. lentiscus* leaves especially those from areas of the southern Mediterranean basin are scarce. To the best of our knowledge, there are no reported studies on the phytochemical composition and antioxidant activity of *P. lentiscus* berries oilcake. A survey carried out by the National Institute of Agronomic Research of Algeria (INRAA) with herbalists from the northern area of the country revealed that the lentisk is most commonly marketed in the form of oil, herbal teas, and soap. The herbalists claim that lentisk oil from the Jijel region was the most appreciated by consumers. Hence, the present work aimed to evaluate the major constituents of *P. lentiscus* leaves and berries oilcake extracts collected from two sites, Mechat and Bordj-Ali, in the province of Jijel (Figure 1) and to investigate their antioxidant activity.

Materials and Methods

Chemicals and Reagents

All reagents were of analytical grade. Folin Ciocalteu's reagent, gallic acid, sodium carbonate, ethanol, 2,2-diphenyl-1-picrylhydrazyl, trichloro-acetic acid, butanol, quercetin, hydrogen peroxide and ammonium molybdate were purchased from Sigma-Aldrich (St. Louis, USA). Sulfuric acid and hydrochloric acid were from Cheminova internacional, SA (Madrid, Spain). Acetic acid was from Sigma-Aldrich, Sleeze, Germany. Aluminium trichloride, ascorbic acid were purchased from Fluka (Buchs, Germany). Potassium ferricyanide, ferric chloride, sodium dihydrogen phosphate and sodium hydrogen phosphate were from Biochem Chemopharma (Quebec, Canada). Methanol was purchased from Merck KGaA (Darmstadt, Germany). Petroleum ether was from Sigma-Aldrish, Germany. Sodium acetates was purchased from Honeywell, Fluka, Germany.

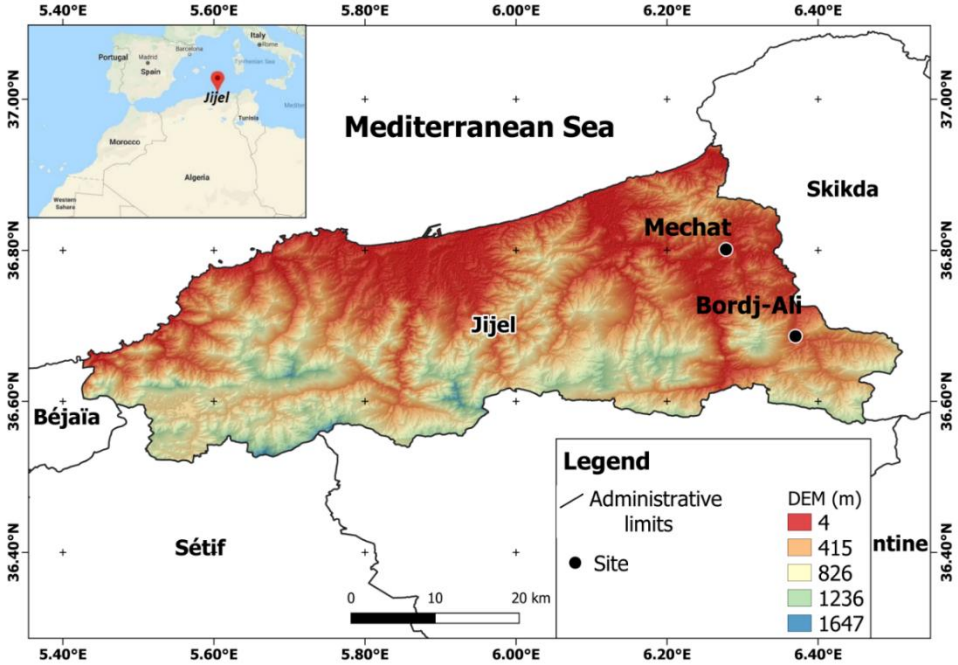

Figure 1. Geolocalization of lentisk collection sites in Jijel province

Plant Material and Sample Preparation

P. lentiscus berries and leaves (Figure 2) were harvested from two locations, the first site "Mechat" is situated at an altitude of 70 m with the following geographical coordinates: 36°48'04.2"N; 6°16'40.0"E. The second site "Bordj-Ali" is situated at a higher altitude (180 m) with the geographical coordinates: 36°41'11.6"N; 6°22'13.7"E. Both sites belong to the province of Jijel (Algeria). The botanical identification was performed by the Phytogenetic Resources Research Division, Algeria's National Institute of Agronomic Research. Fresh leaves were air-dried in shade at room temperature and the fruits were frozen at -20°C until used.

Extraction Procedure

Dried plant leaves were ground to a fine powder (diameter < 0.5 mm) using a laboratory blender (IKA, USA). The fruits were crushed and then defatted using Soxhlet with petroleum ether as a solvent to obtain in the end the fixed oil of lentisk and its oilcake (Figure 2). Samples of leaf powder or fruit oilcake (1 g), were macerated for 24 h, at room temperature, in 10 mL of methanol. The extract solutions were filtered, and then concentrated to dryness in a reduced pressure rotary evaporator (Büchi, Germany) set at 40°C (Amessis-Ouchemoukh et al., 2014). Dry extracts were re-suspended in methanol and stored at − 20°C until used.

Phytochemicals Contents Estimation

Total phenolic content

The Folin–Ciocalteu reagent was used to assay the total phenolic content (TPC), according to the method of Singleton and Rossi (1965). An aliquot of diluted sample extract (0.3 mg/mL) was reacted with 500 µL of the Folin–Ciocalteu reagent and 6 mL of water. The mixture was shaken and allowed to stand for 5 min, before adding 1.5 mL of Na_2CO_3 (20%). A 1.9 mL volume of distilled water was added and properly mixed. After 2 hours of incubation in the dark, the absorbance was measured at 760 nm using UV-2005 spectrophotometer (Selecta, Spain) against the blank solution. Through a calibration curve created with gallic acid ($y = 0.009x + 0.017$; $R^2 = 0.997$), the total phenolic content of plant extracts was represented as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW). Data were reported as mean ± standard deviation for at least three replications.

Total flavonoid content (TFC)

Aluminium chloride was used to determine total flavonoids according to the protocol of Abdel-Hameed, (2009) with a few changes. Quercetin was used as a reference compound. Briefly, 1 mL of each sample solution (0.3 mg/mL) was combined with an equal amount of aluminum trichloride (2%) and a droplet of acetic acid. This was followed by adding methanol to the mixture to make it 5 mL. After 40 min, the absorption was determined at 415 nm. Blank was prepared from all reagents without the samples. Concentrations of flavonoids were deduced from the prepared standard curve ($y = 0.0159x + 0.0052$; $R^2 = 0.999$) and expressed as mg quercetin equivalent per gram dry weight (QE/g DW). All determinations were carried-out in triplicates.

Flavonols content

For this test, the Adedapo et al., (2008) proposed method was operated for estimating the total flavonols in plant extracts. To 2 mL of sample, 2 mL of ethanolic $AlCl_3$ (2%) and 3 mL sodium acetate (50 g/L) solutions were added. The mixture was shaken and incubated for 2.5 h at 20°C. After reading the absorbance at 440 nm, total flavonols content was quantified through the quercetin's calibration curve ($y = 0.0249x + 0.0089$; $R^2 = 0.999$) and reported as milligrams of quercetin equivalents per gram of dry weight (mg EQ/g DW).

Determination of proanthocyanidins content

The concentration of proanthocyanidins was determined by butanol–HCl assay (Maksimovíc et al., 2005). Extracts of lentisk (0.5 mL) were mixed with 3 mL of butanol–HCl (95:5; v/v) and 0.1 mL of 2% ferric reagent (2% ferric ammonium sulfate in 2M HCl). Test tube mixtures were vortexed and put into a boiling water bath for 60 min. After cooling, absorbance was recorded at 530 nm (Amessis-Ouchemoukh et al., 2014) against a blank containing 0.5mL of solvent instead of the extract. Proanthocyanidins were expressed as mg leucoanthocyanidin/g of dry plant material, assuming that the specific absorbance of leucoanthocyanidin was 460. All measurements were done in triplicate

Determination of anthocyanins content

The extracts of fruit oilcake or leaves were obtained by macerating 5 g of the ground material with 50 mL of 70% ethanol solution with 1.5 $mol \cdot L^{-1}$ HCl (85:15, v/v). The suspension was allowed to stand in the absence of light and under refrigeration (4 ± 1°C). After 24 hours, the samples were centrifuged using 2-6E centrifuge (Sigma, Germany) and the recovered volume was completed to 50 mL (Vieira et al., 2018). For the anthocyanin concentration calculations were taken into account the dilution factor and the cyaniding-3-galactoside coefficient of extinction 98.2 (Fuleki and Francis, 1968).

Evaluation of the Antioxidant Activity

Four methods were used for the evaluation of the antioxidant properties of lentisk extracts: 1,1-diphenylpicrylhydrazyl radical scavenging activity, hydrogen peroxide (H_2O_2) radical scavenging activity, reducing power activity, and total antioxidant capacity. All experiments were performed in triplicate.

Determination of the DPPH free radical scavenging activity

The ability of lentisk extracts to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated as previously described by Mazari et al., (2018). Various dilutions of *P. lentiscus* extracts or control ascorbic acid (2 mL) were mixed with 2 mL of a 0.2 mM methanolic solution of DPPH. Reaction mixtures were incubated in a dark place at room temperature for 30 minutes. Then the absorbance was measured at 517 nm. The disappearance of DPPH was recorded and the percent inhibition of the DPPH radicals by the sample was calculated as follows:

$$\text{DPPH radical scavenging (\%)} = [(Ac\text{-}As) \div Ac] \times 100$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

Determination of Total Antioxidant Capacity

Total antioxidant capacity (TAC) in the *P. lentiscus* extracts was determined using the phosphomolybdenum method as described by Prieto et al., (1999). Each sample

(0.6 mL) was mixed with 6 mL reagent solution [sodium phosphate (28 mM), sulfuric acid (0.6 M) and ammonium molybdate (4 mM)]. The tubes were incubated for 90 min at 95°C. After cooling, the absorbance was read at 695 nm against the blank where (6 mL reagent solution was mixed with 0.6 mL of methanol) incubated within the same basic conditions as the samples. The ascorbic acid (AA) was used as standard and the TAC assay was expressed as milligram equivalents of ascorbic acid per gram of dry weight (mg EAA/g DW).

Scavenging activity against hydrogen peroxide

Hydrogen peroxide (H_2O_2) scavenging activity was estimated by the method of Ruch et al., (1989). A 40 mM solution of H_2O_2 was prepared in a phosphate buffer (pH 7.4). Two milliliters of plant extracts were mixed with 1.2 mL of H_2O_2 solution. The final extract concentration in the reaction media was 60 µg/mL. A blank solution was prepared in the same way but without H_2O_2. After incubation of the mixture for 10 min, the absorbance was recorded at 230 nm. The scavenging activity was calculated using the following formula:

$$H_2O_2 \text{ radical scavenging activity } (\%) = [(Ac\text{-}At) \div Ac] \times 100$$

Where Ac is the absorbance of the control and At is the absorbance of the extract.

Ferric reducing power

The reducing power was determined according to a procedure based on the method of Oyaizu, (1986). Aliquots (2 mL) of different dilutions of lentisk leaves and berries oilcake extracts (0.01-0.2 mg/mL) or standard ascorbic acid (2.5~40 µg/mL) were added phosphate buffer (2 mL, 0.2 M, pH 6.6) and mixed with potassium ferricyanide (2mL, 1%). Then, the mixtures were incubated at 50°C for 20 min. The reaction was terminated by trichloroacetic acid solution (2 mL, 10%) and centrifuged at 3,900 rpm for 10 min. Two milliliters of the supernatant was taken out immediately and added 2 mL of methanol and mixed with ferric chloride (0.5 mL, 0.1%). The absorbance was measured at 700 nm against a blank. Three replicates were performed on control (ascorbic acid) and each tested sample.

Statistical Analysis

All the statistics were performed using the XLSTAT software for Windows. Mean values obtained for the variables studied in the different groups were compared by one-way ANOVA, assuming that there were significant differences among them when the statistical comparison gives $P<0.05$. To correctly assess the correlation between the contents of phytochemicals and the antioxidant activity estimated by the parameters SC_{50} and EC_{50}, the constants K_{SC50} and K_{EC50}, which are the inverted fraction of SC_{50} and EC_{50}, were calculated and used in the correlation study (Table 3).

Results and Discussion

Phytochemical Content Evaluation

Total phenolic, flavonoids, and flavonols contents

Table 1 illustrates total phenolics, total flavonoids, and flavonols contents in leaves and berries oil cake of both study sites. The site of collection has a significant effect ($p < 0.05$) on the content of total polyphenols. The content of phenolic compounds in berries oilcake was lower than in leaves extracts. The highest content was recorded for leaves extract from Mechat (147.80 mg GAE/g DW), while this value drops to 143.39 mg GAE/g DW for leaves extract from Bordj-Ali.

In the case of berries oil cakes, the higher content was observed for the sample from Bordj-Ali with a value of 21.57 mg GAE/g, versus 10.57 mg GAE/g for the sample collected from the site of Mechat. Samples from the Bordj-Ali site exhibited the highest flavonoids content both in leaves (3.14 mg QE/g DW) and berries oil cake (1.30 mg QE/g); whereas flavonols amount was higher in leaves of Mechat (3.14 mg QE/g DW) than Bordj-Ali (2.74 mg QE/g DW).

The range of polyphenols content is in good agreement with the results reported by Atmani et al., (2009); Dahmoune et al., (2014); Yosr et al., (2018) and Barbouchi et al., (2020). However, it is beyond the values reported by Cherbal et al., (2012); Remila et al., (2015) and Mehenni et al., (2016). This discrepancy could be attributed to differences in the analytical protocols on one hand and some other factors such as gender and the cycle growth stages of the plant on the other hand. Indeed, in the study reported by Yosr et al., (2018), they found that male vegetative organs had significantly higher phenolic contents than females. The phenolic content they reported varied from 87 to 178.5 mg GAE/g DW for female leaves and from 106.5 to 209.5 mg GAE/g DW for male leaves in the late flowering stage (LF) and dormancy period (DP), respectively. They stipulated that the high production of phenolic compounds could be a part of the putative defense strategy against herbivores, pathogens, and drought resistance as well as a strategy to face more than females lower temperatures of winter (Yosr et al., 2018). Moreover, increased accumulation of antioxidants, such as flavonoids and anthocyanins, in plants growing in highlands could be a mechanism of adaptation to adverse environmental conditions and as a component of the response to a higher level of UV radiation or lower temperatures (Chanishvili et al., 2007).

Remila et al., (2015) used the combined procedure UPLC-DAD and electrospray ion ionization mass spectrometry to identify the polyphenols of *P. lentiscus* extract. They identified eleven phenolic compounds in the leaves extract. The identified compounds were phenolic acid derivatives: glucogallin, gallic acid, galloyl quinic acid derivatives, and flavonols derivatives which were all conjugated with a sugar moiety: myricetin-rutinoside, myricetin-glucoside, quercetin-rutinoside, myricetin-rhamnoside, quercetin-glucoside, and quercetin-rhamnoside. In another study, using the HPLC-ESI-QTOF-MS method, Rodríguez-Pérez et al., (2013) identified 46 compounds and classified them into 4 groups: flavonoids, hydroxycinnamic acid derivatives, phenolic acid derivatives, and other polar compounds. Twenty compounds were identified as flavonoids: (+)-Catechin, D-gallocatechin, luteolin, and other compounds most of which were myricetin-glycosides, quercetin glycosides, and kaempferol-glycosides. They reported that two molecules were described for the first time in *P. lentiscus*: quercitrin gallate that is responsible for the prevention of diabetic complications and improvement of intestinal inflammatory response and glabrol, which have been reported to decrease cholesterol absorption and have been associated with the modulation of the expression of a group of genes that regulate glucose and lipid metabolism.

Anthocyanins and Proanthocyanidins contents

Based on the results of the assessment (Table 1) anthocyanins were present in the berries oilcake of *P. lentiscus* with an estimated value of 18.16 ± 1.83 and 11.16 ± 0.85 mg/g for the samples from Bordj-Ali and Mechat sites, respectively. Leaves were almost totally devoid of anthocyanins: 0.019 ± 0.002 and 0.014 ± 0.001 mg/g for the samples from Mechat and Bordj-Ali Sites, respectively.

According to several studies, the variation in anthocyanin levels depends on genetic and physiological factors such as genotype and degree of maturation (Vieira et al., 2018). The accumulation of anthocyanins is influenced by environmental factors including light, temperature, plant nutrition, and pathogen attacks. Indeed, light exerts two opposite effects on anthocyanins: in vivo, within the plant, it promotes their biosynthesis, whereas, in vitro in extracts or products it accelerates their degradation (Malien-Aubert and Amiot-Carlin, 2006). The increased anthocyanin may indicate that the anthocyanin screen plays a key role in protecting plants from high-level UV radiations in high mountains (Chanishvili et al., 2007). Longo et al., (2007) reported that the major anthocyanin of *P. lentiscus* berries has been identified as cyanidin-3-O-glucoside, while delphinidin-3-O-glucoside and cyaniding-3-O-arabinoside have been found in minor quantities. Interest in anthocyanin pigments is growing due to their use as a natural food coloring and especially as antioxidant and anti-inflammatory agents. A wide range of nutraceutical and pharmaceutical effects of anthocyanins, including neuroprotective effect, antiobesity, antidiabetes, anticancer, antioxidant, antimicrobial, cardiovascular, and visual health claim, were extensively summarized in the review of Khoo et al., (2017).

The proanthocyanin contents of the methanolic extracts of *P. lentiscus* (leaves and berries oilcake) were determined by the Butanol-HCl method. The results were expressed in mg leucoanthocyanidins equivalent/gram are shown in Table 2. The highest value in proanthocyanidins was noted for the leaves of Bordj-Ali and Mechat site, 128.60 ± 1.76 and 86.76 ± 2.01 mg eq. L/g DW, respectively. The obtained values are higher than those reported by Amessis-Ouchemoukh et al., (2014), for leaves collected from Bejaia city (Algeria) where they reported proanthocyanidins content of 39.29 ± 0.61 mg CE/g DW. In the case of berries oilcake, the lower value was recorded for the sample from the site of Mechat: 4.28 ± 0.24 mg eq. L/g while, the sample from the Bordj-Ali site showed a significantly higher content (14.62 ± 0.22 mg eq. L/g). These results suggest that *P. lentiscus* leaves contained a substantial amount of condensed tannins (Figure 3).

Wu et al. (2004) characterized anthocyanins in some cultivars of black and red currant, gooseberries, chokeberry, and elderberry. They detected thirty-one different anthocyanins. Among the studied berries, chokeberry had the highest total anthocyanins concentration (1480 mg/100 g FW). Total proanthocyanidins concentrations ranged from 23 to 664 mg/100 g FW in elderberry and chokeberry, respectively. Procyanidin or prodelphinidin polymers being the predominant components (> 65%) in most of the berries. One of the most important characteristics of this group of bioactive components is that the intake of A-type proanthocyanidins-rich food is associated with the prevention of urinary tract infections (Krueger et al., 2013).

Evaluation of Antioxidant Activities

Determination of the DPPH free radical scavenging activity

The antioxidant activity of *P. lentiscus* methanolic extracts was assessed through the DPPH free radical scavenging test. The results obtained are given in Table 2. The DPPH radical scavenging activity of *P. lentiscus* leaves and berries oilcake extracts and ascorbic acid, which was used as a reference antioxidant, was evaluated by the IC_{50} parameter that is the extract concentration capable of trapping 50% of the DPPH radicals in the reaction media. A great and significantly higher radical scavenging activity was observed for leaves extracts of *P. lentiscus* of both sites (IC_{50}: 10.46 ± 1.05 µg/mL for Mechat and 11, 82 ± 1.81 µg/mL for Bordj-Ali). These values were statistically similar in strength to that of the reference ascorbic acid (Table 2). Methanolic extract of berries oilcake from Mechat showed the weakest DPPH radical scavenging activity among the tested samples (IC_{50}: 45.94 ± 4.04 µg/mL); while Bordj-Ali berries oilcake extract exhibited a better DPPH free radical scavenging activity (IC_{50}: 29.13 ± 4.05 µg/mL) than that of Mechat site. These results are higher than those reported by Bampouli et al., (2014). They tested different extraction procedures (Soxhlet extraction, microwave-assisted extraction, and ultrasound-assisted extraction) which released IC_{50} values ranging from 37.13 ~ 84.79 µg/mL. Barbouchi et al. (2020) studied the DPPH scavenging activity of different extracts of lentisk collected from two regions in Morocco. They reported the IC_{50} values of 1.13 and 0.57 mg/mL for the methanolic extracts of leaves. Dahmoune et al., (2015) proceeded for the extraction through three techniques (ultrasound-assisted extraction, accelerated solvent extraction, and conventional solvent extraction) they obtained IC_{50} values ranging between 18.74 ~ 32.77 µg/mL. Our results are in good agreement with those of Yosr et al., (2018), who tested lentisk samples of different maturation stages and found mean values laying between 4.9 ~ 6.4 µg/mL. The same trend was observed in the study of Gardeli et al., (2008), who assessed the DPPH radical scavenging activity in three different stages (February, May, and August). They obtained IC_{50} values ranging from 5.09 ~ 11.0 mg/L. They concluded that the highest antioxidant activities were observed in samples of the full flowering stage.

Scavenging activity against hydrogen peroxide

The hydrogen peroxide (H_2O_2) scavenging activity was tested on *P. lentiscus* extracts. Leaf extracts were more efficient H_2O_2 radical scavengers, particularly leaf extract of Bordj-Ali was significantly higher than those of Mechat leaf and berries oilcake extracts of both study sites (Table 2). Indeed, 60µg/mL of Mechat and Bordj-Ali leaf extracts induced the inhibition of 20.23 and 25.92% of H_2O_2 free radicals, respectively. This result is slightly below those of Cherbal et al., (2012) who observed an H_2O_2 inhibition rate of 37.04% when applying hydromethanolic extract of *P. lentiscus* leaves at 50µg/mL. Atmani et al., (2009) reported a scavenging activity rate ranging from 22.5 ~ 75.11% for leaves extracted with different solvents. They speculated that the observed scavenging activity could be exerted by phenolic compounds. Moreover, to examine the impact of oxidative stress on cell viability, Remila et al., (2015) used H_2O_2 as an oxidant agent to induce ROS formation in multiple cell types. They found that pre-treatment of THP-1 cells with *P. lentiscus* extracts for 24 h strongly inhibited H_2O_2 damage. They suggested that those extracts possess a high potential to scavenge intracellular ROS.

Table 1. Phytochemicals content of *Pistacia lentiscus* leaves and berries oilcake.

	Polyphenols (mg GAE/g)	Flavonoids (mg QE/g)	Flavonols (mg QE/g)	Proanthocyanidins (mg LAE/g)	Anthocyanins (mg/g)
LSM	147.80±1.79^{a}	4.74±0.20^{b}	3.14±0.15^{a}	86.83±2.02^{b}	0.02±0.00^{c}
LSB	143.39±1.05^{b}	5.46±0.16^{a}	2.74±0.30^{b}	139.12±1.90^{a}	0.01±0.00^{c}
CSM	10.57±0.50^{d}	0.68±0.01^{d}	0.82±0.05^{d}	4.28±0.24^{d}	11.16±0.86^{b}
CSB	22.18±1.76^{c}	1.31±0.07^{c}	1.30±0.01^{c}	14.62±0.22^{c}	18.16±1.83^{a}

Values were expressed as the mean of triplicate determinations ± standard deviation, Values with the same letter within the same column did not differ significantly from each other according to the LSD test at P<0.05, LSM: leaves of Mechat; LSB: leaves of Bordj-Ali; CSM: berries oilcake of Mechat; CSB: berries oilcake of Bordj-Ali.

Table 2. Antioxidant activity of *Pistacia lentiscus* berries oilcake and leaves.

	DPPH free radical scavenging activity SC_{50}	K_{SC50}	Ferric Reducing power EC_{50}	K_{EC50}	Total antioxidant capacity	H_2O_2 radical scavenging activity
	(µg/mL)		(µg/mL)		(mg EAA/g)	(%)
LSM	10.46±1.06^{a}	9.63±0.95^{a,b}	28.59±2.19^{a}	3.51±0.26^{a}	104.07±2.39^{b}	20.23±0.03^{b}
LSB	11.82±1.82^{a}	8.59±1.30^{b}	28.08±2.85^{a}	3.59±0.38^{a}	159.39±4.55^{a}	25.92±1.33^{a}
CSM	47.16±6.07^{c}	2.14±0.28^{c}	229.21±6.25^{c}	0.44±0.01^{c}	6.13±0.46^{d}	4.23±0.15^{e}
CSB	29.13±4.05^{b}	3.47±0.45^{c}	44.91±3.13^{b}	2.23±0.16^{b}	14.96±0.03^{c}	15.93±1.67^{c}
	7.92±1.21^{a,*}	12.84±2.15^{a,*}	32.63±3.22^{a,*}	3.09±0.32^{a,*}		9.15±0.97^{d,*}

Values were expressed as the mean of triplicate determinations ± standard deviation, Values with the same letter within the same column did not differ significantly from each other according to the LSD test at P<0.05, LSM: leaves of Mechat; LSB: leaves of Bordj-Ali; CSM: berries oilcake of Mechat; CSB: berries oilcake of Bordj-Ali. * Control: Ascorbic acid.

Table 3. Correlation matrix Person (n) between phytochemicals and antioxidant activity of *P. lentiscus* extracts:

Variables	Polyphenols	Flavonoids	Flavonols	Proanthocyanidins	Anthocyanins	K_{SC50}	K_{EC50}	H_2O_2 Scav.	TAC
Polyphenols	1								
Flavonoids	0,986	1							
Flavonols	0,978	0,970	1						
Proanthocyanidins	0,933	0,945	0,869	1					
Anthocyanins	-0,922	-0,895	-0,843	-0,857	1				
K_{SC50}	0,970	0,950	0,945	0,873	-0,855	1			
K_{EC50}	0,889	0,909	0,897	0,840	-0,658	0,907	1		
H_2O_2 Scav.	0,839	0,887	0,844	0,878	-0,566**	0,819	0,959	1	
TAC	0,943	0,956	0,879	0,998	-0,873	0,887	0,846	0,873	1

All values are different from 0 at a level of significance ($\alpha = 0.05$). only one value (-0.566) is not significant.

Ferric Reducing power

The reducing power of *P. lentiscus* leaves and berries oilcake methanolic extracts are presented in Figure 4. The reducing power of the extracts increased concomitantly with their concentration. Leaves extracts of both study sites exhibited the highest reducing power, whereas berries oilcake extract from Mechat showed the weakest reducing power. The reducing power strength was estimated through the EC_{50} parameter that is the required concentration to induce a spectrophotometric absorbance of 0.5. There were no significant difference between the reducing power of leaf extracts of both study sites. Their EC_{50} values were statistically similar to that of the reference ascorbic acid. The reducing power of Bordj-Ali berries oilcake extract (EC_{50}: 44.91 µg/mL) was almost equal to half of the strength of leaves extracts whereas Mechat berries oilcake extract (EC_{50}: 229.21 µg/mL) was eight folds less powerful than leaves extract (Table 2). The observed reducing power of *P. lentiscus* leaves extracts was higher than that stated by Atmani et al., (2009) who reported the absorbance values of 0.91 and 0.99 when applying 100µg/mL of aqueous fractions issued from hexane and chloroform partitions, respectively; and those of Beghlal et al., (2016), who assessed the reducing power of the ethanolic extract (50 µg/mL) which provided an absorbance value of 0.425.

Determination of Total Antioxidant Capacity

The total antioxidant capacity (TAC) results obtained by the phosphomolybdenum method were presented in Table 2. The result showed a significant difference (P<0.05) in the TAC between all extracts of *P. lentiscus*. Methanolic extracts of leaves and berries oilcake from Bordj-Ali recorded the greatest TAC compared to those from Mechat. The methanolic extract of leaves from Bordj-Ali, showed the highest TAC with 159.39 mg EAA/g, while the extract of berries oilcake from Mechat had the least amount of TAC with 6.13 mg EAA/g (Table 2). In their investigation, Barbouchi et al., (2020) reported TAC values ranging from 239.98 ~254.58 and from 207.91 ~ 221.74 mg EAA/g crude extracts for leaves and berries, respectively.

Overall, the tests of antioxidant activity deployed in this study, a very significant correlation was established between the phytochemical content and the antioxidant capacity with a factor of correlation included between 0.839 ~ 0.998 (Table 3) implying that the richer is the extract in phytochemicals the greater is the antioxidant capacity. This may be explained by the antioxidant effect where active phytochemical compounds exert a role by their reaction with free radical molecules through electron or hydrogen transfer mechanism thus inducing their neutralization. Moreover, since the extract included a

mixture of different kinds of antioxidant components, they could have exerted a synergistic effect that may have enhanced the antioxidant power.

Figure 2. Lentisk shrub and the collected and treated samples: A. Lentisk shrub; B. and C. Lentisk berries; D. Oilcake; E. dried lentisk leaves.

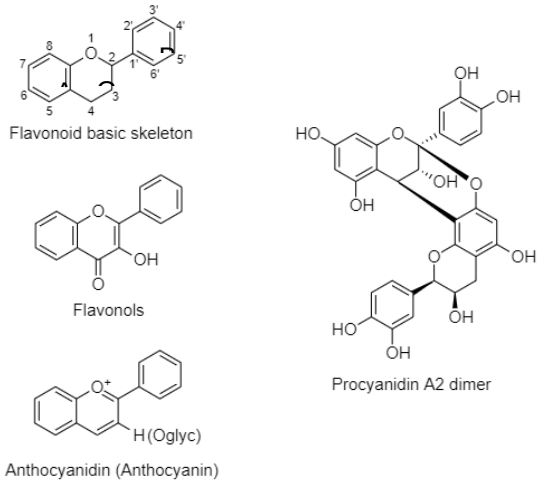

Figure 3. Chemical structure of some Flavonoids and condensed tannins
(Source: Kelm et al., 2005; Panche et al., 2016).

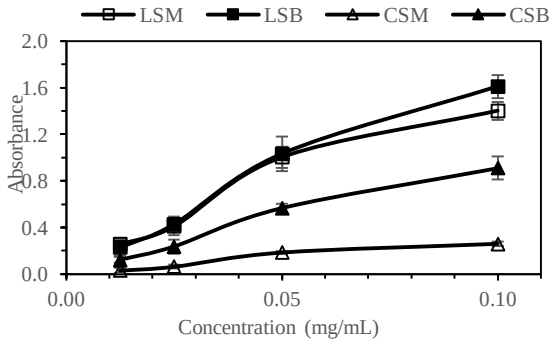

Figure 4. Ferric reducing power of *Pistacia lentiscus* leaves and berries oilcake extracts.
Different extracts were symbolized as follow: LSM, leaves extract from Mechat; LSB, leaves extract from Bordj-Ali; CSM, berries oilcake extract from Mechat; berries oilcake extract from Bordj-Ali.

Conclusion

This study focused on the evaluation of the phytochemical composition and antioxidant activity of the extracts of lentisk leaves and berries oilcake from two sites in the province of Jijel. Results showed the high content of these extracts and in particular those of leaves in polyphenols and proanthocyanidins (condensed tannins). The extracts also contained appreciable amounts of flavonoids and flavonols. In the aim of valuing post-agro-manufacturing wastes, the chemical composition and the antioxidant activity of lentisk berries oilcake was investigated. The oilcake of the lentisk berries contained non-negligible amounts of polyphenols and a substantial amount of natural coloring dyes (anthocyanin), very useful substances in the formulations of the food industry as food additives.

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Compliance with Ethical Standards

The authors declare no conflict of interest.

This article does not any studies with human participants or animals performed by any of the authors.

References

Abdel-Hameed ESS. 2009. Total phenolic contents and free radical scavenging activity of certain Egyptian Ficus species leaf samples. Food Chemistry, 114(4): 1271–1277. doi:10.1016/j.foodchem.2008.11.005

Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. 2008. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of Calpurnia aurea. BMC Complementary and Alternative Medicine, 8: 1–8. doi: 10.1186/1472-6882-8-53

Ain-lhout F, Díaz Barradas MC, Zunzunegui M, Rodríguez H, García Novo F, Vargas MA. 2004. Seasonal differences in photochemical efficiency and chlorophyll and carotenoid contents in six Mediterranean shrub species under field conditions. Photosynthetica, 42(3): 399–407. extension://mbcgpelmjnpfbdnkbebdlfjmeckpnhha/enhanced-reader.html?openApp&pdf=http%3A%2F%2Fps.ueb.cas.cz%2Fpdfs%2Fphs%2F2004%2F03%2F15.pdf

Amessis-Ouchemoukh N, Madani K, Falé PLV, Serralheiro ML, Araújo MEM. 2014. Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Industrial Crops and Products, 53: 6–15. doi: 10.1016/j.indcrop.2013.12.008

Ammari M, Othman H, Hajri A, Sakly M, Abdelmelek H. 2018. Pistacia lentiscus oil attenuates memory dysfunction and decreases levels of biomarkers of oxidative stress induced by lipopolysaccharide in rats. Brain Research Bulletin, 140: 140–147. doi: 10.1016/j.brainresbull.2018.04.014

Atmani D, Chaher N, Berboucha M, Ayouni K, Lounis H, Boudaoud H, Debbache N, Atmani D. 2009. Antioxidant capacity and phenol content of selected Algerian medicinal plants. Food Chemistry, 112(2): 303–309. doi: 10.1016/j.foodchem.2008.05.077

Azaizeh H, Halahleh F, Abbas N, Markovics A, Muklada H, Ungar ED, Landau SY. 2013. Polyphenols from Pistacia lentiscus and Phillyrea latifolia impair the exsheathment of gastro-intestinal nematode larvae. Veterinary Parasitology, 191(1–2): 44–50. doi: 10.1016/j.vetpar.2012.08.016

Balan KV, Prince J, Han Z, Dimas K, Cladaras M, Wyche JH, Sitaras NM, Pantazis P. 2007. Antiproliferative activity and induction of apoptosis in human colon cancer cells treated in vitro with constituents of a product derived from Pistacia lentiscus L. var. chia. Phytomedicine, 14(4): 263–272. doi: 10.1016/j.phymed.2006.03.009

Bampouli A, Kyriakopoulou K, Papaefstathiou G, Louli V, Krokida M, Magoulas K. 2014. Comparison of different extraction methods of Pistacia lentiscus var. chia leaves: Yield, antioxidant activity and essential oil chemical composition. Journal of Applied Research on Medicinal and Aromatic Plants, 1(3): 81–91. doi: 10.1016/j.jarmap.2014.07.001

Barbouchi M, Elamrani K, El Idrissi M, Choukrad M. 2020. A comparative study on phytochemical screening, quantification of phenolic contents and antioxidant properties of different solvent extracts from various parts of Pistacia lentiscus L. Journal of King Saud University - Science, 32(1): 302–306. doi: 10.1016/j.jksus.2018.05.010

Beghlal D, El Bairi K, Marmouzi I, Haddar L, Mohamed B. 2016. Phytochemical, organoleptic and ferric reducing properties of essential oil and ethanolic extract from Pistacia lentiscus (L.). Asian Pacific Journal of Tropical Disease, 6(4): 305–310. doi: 10.1016/S2222-1808(15)61035-0

Bellakhdar J. 2003. Le Maghreb à travers ses plantes: plantes, productions végétales et traditions au Maghreb. Editions Le Fennec.

Boudieb K, Ait Slimane-Ait Kaki S., Amellal-Chibane H. 2019. Traditional uses, phytochemical study and morphological characterization of Pistacia lentiscus L. fruits from three areas of northern Algeria. Journal of Applied Biosciences, 135(1): 13788. doi: 10.4314/jab.v135i1.5

Chanishvili S, Badridze G, Rapava L, Janukashvili N. 2007. Effect of altitude on the contents of antioxidants in leaves of some herbaceous plants. Russian Journal of Ecology, 38(5): 367–373. doi: 10.1134/S1067413607050128

Cherbal A, Kebieche M, Madani K, El-Adawi H. 2012. Extraction and valorisation of phenolic compounds of leaves of Algerian Pistacia lentiscus. Asian Journal of Plant Sciences, 11(3): 131–136. doi: 10.3923/ajps.2012.131.136

Cheurfa M and Allem R. 2015. Study of hypocholesterolemic activity of algerian pistacia lentiscus leaves extracts in vivo. Revista Brasileira de Farmacognosia, 25(2): 142–144. doi: 10.1016/j.bjp.2015.02.011

Dahmoune F, Remini H, Dairi S, Aoun O, Moussi K, Bouaoudia-Madi N, Adjeroud N, Kadri N, Lefsih K, Boughani L, Mouni L, Nayak B, Madani K. 2015. Ultrasound assisted extraction of phenolic compounds from P. lentiscus L. leaves: Comparative study of artificial neural network (ANN) versus degree of experiment for prediction ability of phenolic compounds recovery. Industrial Crops and Products, 77: 251–261. doi: 10.1016/j.indcrop.2015.08.062

Dahmoune F, Spigno G, Moussi K, Remini H, Cherbal A, Madani K. 2014. Pistacia lentiscus leaves as a source of phenolic compounds: Microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Industrial Crops and Products, 61: 31–40. doi: 10.1016/j.indcrop.2014.06.035

de Lanfranchi F, Bui Thi M, Girard M. 1999. La fabrication d'huile de lentisque (Linsticu ou chessa) en Sardaigne. Journal d'agriculture Traditionnelle et de Botanique Appliquée, 41(2): 81–100. doi: 10.3406/jatba.1999.3712

Dellai A, Souissi H, Borgi W, Bouraoui A, Chouchane N. 2013. Antiinflammatory and antiulcerogenic activities of Pistacia lentiscus L. leaves extracts. Industrial Crops and Products, 49: 879–882. doi: 10.1016/j.indcrop.2013.07.010

Foddai M, Kasabri V, Afifi FU, Azara E, Petretto GL, Pintore G. 2015. In vitro inhibitory effects of Sardinian Pistacia lentiscus L. and Pistacia terebinthus L. on metabolic enzymes: Pancreatic lipase, α-amylase, and α-glucosidase. Starch/Staerke, 67(1–2): 204–212. doi: 10.1002/star.201400068

Dimas K, Hatziantoniou S, Wyche JH, Pantazis P. 2009. A Mastic gum extract induces suppression of growth of human colorectal tumor xenografts in immunodeficient mice. In vivo (Athens, Greece) 23(1): 63-68.

Fuleki T and Francis FJ. 1968. Quantitative methods for anthocyanins. 1. Extraction and determination of total anthocyanin in croranberries. Journal of Food Science, 33(1): 72–77. doi: 10.1111/j.1365-2621.1968.tb00887.x

Gardeli C, Vassiliki P, Athanasios M, Kibouris T, Komaitis M. 2008. Essential oil composition of Pistacia lentiscus L. and Myrtus communis L.: Evaluation of antioxidant capacity of methanolic extracts. Food Chemistry, 107(3): 1120–1130. doi: 10.1016/j.foodchem.2007.09.036

Kelm MA, Hammerstone JF, Schmitz HH. 2005. Identification and quantitation of flavanols and proanthocyanidins in foods: How good are the datas? Clinical and Developmental Immunology, 12(1): 35–41. doi: 10.1080/10446670410001722177

Khoo HE, Azlan A, Tang ST, Lim SM. 2017. Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. Food and Nutrition Research, 61(1): 1361779. Doi: 10.1080/16546628.2017.1361779

Krueger CG, Reed JD, Feliciano RP, Howell AB. 2013. Quantifying and characterizing proanthocyanidins in cranberries in relation to urinary tract health. Analytical and Bioanalytical Chemistry, 405(13): 4385–4395. doi: 10.1007/s00216-013-6750-3

Landau S, Azaizeh H, Muklada H, Glasser T, Ungar ED, Baram H, Abbas N, Markovics A. 2010. Anthelmintic activity of Pistacia lentiscus foliage in two Middle Eastern breeds of goats differing in their propensity to consume tannin-rich browse. Veterinary Parasitology, 173(3): 280–286. doi: 10.1016/j.vetpar.2010.07.006

Longo L, Scardino A, Vasapollo G. 2007. Identification and quantification of anthocyanins in the berries of Pistacia lentiscus L., Phillyrea latifolia L. and Rubia peregrina L. Innovative Food Science and Emerging Technologies, 8(3): 360–364. doi: 10.1016/j.ifset.2007.03.010

Maksimovíc Z, Maleňcíc D, Kovăcevíc N. 2005. Polyphenol contents and antioxidant activity of Maydis stigma extracts. Bioresource Technology, 96(8): 873–877. doi: 10.1016/j.biortech.2004.09.006

Malien-Aubert C and Amiot-Carlin MJ. 2006. Pigments phénoliques - Structures, stabilité, marché des colorants naturels et effets sur la santé. In Les polyphénols en Agroalimentaire (pp. 296-333.). Tec & Doc - Lavoisier. http://www.iamm.ciheam.org/ress_doc/opac_css/index.php?lvl=notice_display&id=23719

Manolaraki F, Sotiraki S, Stefanakis A, Skampardonis V. 2010. Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. Parasitology, 137: 685–696. doi: 10.1017/S0031182009991399

Mansour-Djaalab H, Kahlouche-Riachi F, Djerrou Z, Serakta-Delmi M, Hamimed S, Trifa W, Djaalab I, Pacha YH. 2012. In vitro evaluation of antifungal effects of Lawsonia inermis, Pistacia lentiscus and Juglans regia. Int. J. Med. Arom. Plants, 2(2): 263–268.

Markovics A, Cohen I, Muklada H, Glasser TA, Dvash L, Ungar ED, Azaizeh H, Landau SY. 2012. Consumption of Pistacia lentiscus foliage alleviates coccidiosis in young goats. Veterinary Parasitology, 186(3): 165–169. doi: 10.1016/j.vetpar.2011.11.072

Mazari A, Yahiaoui K, Fedjer Z, Mahdeb A. 2018. Physical characteristics, phytochemical content and antioxidant activity of cactus pear fruits growing in Northeast Algeria. Journal of the Professional Association for Cactus Development, 20, 177–195. http://www.jpacd.org/jpacd/article/view/36/23

Mehenni C, Atmani-Kilani D, Dumarçay S, Perrin D, Gérardin P, Atmani D. 2016. Hepatoprotective and antidiabetic effects of Pistacia lentiscus leaf and fruit extracts. Journal of Food and Drug Analysis, 24(3): 653–669. doi: 10.1016/j.jfda.2016.03.002

Mharti FZ, Lyoussi B, Abdellaoui A. 2011. Natural Product Communications Antibacterial Activity of the Essential Oils of Pistacia lentiscus Used in Moroccan Folkloric Medicine. Natural Products Communications, 6(10): 1505–1506. doi: 10.1177/1934578X1100601024

Oyaizu M. 1986. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. The Japanese Journal of Nutrition and Dietetics, 44(6): 307–315. doi: 10.5264/eiyogakuzashi.44.307

Pachi VK, Mikropoulou EV, Gkiouvetidis P, Siafakas K, Argyropoulou A, Angelis A, Mitakou S, Halabalaki M. 2020. Traditional uses, phytochemistry and pharmacology of Chios mastic gum (Pistacia lentiscus var. Chia, Anacardiaceae): A review. Journal of Ethnopharmacology, 254: 1-18. doi: 10.1016/j.jep.2019.112485

Panche AN, Diwan AD, Chandra SR. 2016. Flavonoids: An overview. Journal of Nutritional Science, 5:e47. Doi: 10.1017/jns.2016.41

Prieto P, Pineda M, Aguilar M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a Analytical Biochemistry, 269: 337–341. doi: 10.1037/a0037168

Remila S, Atmani-Kilani D, Delemasure S, Connat JL, Azib L, Richard T, Atmani D. 2015. Antioxidant, cytoprotective, anti-inflammatory and anticancer activities of Pistacia lentiscus (Anacardiaceae) leaf and fruit extracts. European Journal of Integrative Medicine, 7(3): 274–286. doi: 10.1016/j.eujim.2015.03.009

Rodríguez-Pérez C, Quirantes-Piné R, Amessis-Ouchemoukh N, Khodir M, Segura-Carretero A, Fernández-Gutierrez A. 2013. A metabolite-profiling approach allows the identification of new compounds from Pistacia lentiscus leaves. Journal of Pharmaceutical and Biomedical Analysis, 77: 167–174. doi: 10.1016/j.jpba.2013.01.026

Ruch RJ, Cheng SJ, Klaunig JE. 1989. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea. Carcinogenesis, 10(6): 1003–1008. doi: 10.1093/carcin/10.6.1003

Singleton VL and Rossi JA. 1965. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. American Journal of Enology and Viticulture, 16(3): 144–158. http://www.ajevonline.org/content/16/3/144.abstract

Smail-Saadoun N. 2005. Stomata types of Pistacia genus: Pistacia atlantica Desf. ssp. Atlantica and Pistacia lentiscus L. In C. V. (ed.). Oliveira M.M. (ed.) (Ed.), XIII GREMPA Meeting on Almonds and Pistachios (Options Méditerranéennes : Série A. Séminaires Méditerranéens): Vol. n. 63 (pp. 369–371). CIHEAM. http://om.ciheam.org/article.php?IDPDF=5600054%0D

Vieira LM, Marinho LMG, Rocha J de CG, Barros FAR, Stringheta PC. 2018. Chromatic analysis for predicting anthocyanin content in fruits and vegetables. Food Science and Technology, 39(2): 415–422. doi: 10.1590/fst.32517

Wu X, Gu L, Prior RL, McKay S. 2004. Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. Journal of Agricultural and Food Chemistry, 52(26): 7846–7856. doi: 10.1021/jf0486850

Yosr Z, Imen BHY, Rym J, Chokri M, Mohamed B. 2018. Sex-related differences in essential oil composition, phenol contents and antioxidant activity of aerial parts in Pistacia lentiscus L. during seasons. Industrial Crops and Products, 121(February): 151–159. doi: 10.1016/j.indcrop.2018.04.067