Chemical Composition of Leaves and Hull from Pistacia Vera L. an Evaluation of Phenolic Content and Antioxidant Properties of their Extracts

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Research Article

Keywords: Pistacia vera L. chemical characterization, phenolic compounds, antioxidant activity

DOI: https://doi.org/10.21203/rs.3.rs-128147/v1

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Abstract

The pistachio industry in Tunisia generates a high amount of waste every year due to the large pistachio production. This, together with the good properties of the compounds that can be extracted from these by-products, makes it necessary a comprehensive study of their potential. Because of that, the aim of this work was to analyze the chemical characterization of male and female leaves and hull of *Pistacia vera* L. and their ethanol-water extracts. With that purpose, the total phenolic and flavonoid contents of the extracts were determined and their antioxidant activities were investigated using DPPH, ABTS and FRAP methods. The total phenolic content varied from 218 to 533 mg GAE/g DE, and the total flavonoid content varied from 119 to 397 mg CE/g DE. In general, the antioxidant activity showed significant values. The DPPH assay and the ABTS assay were in the range of 332–704 mg TE/g DE and 427 and 1394 mg TE/g DE, respectively. In the case of FRAP, male leaves extract had the best result, being the TE value 808 mg TE/g DE. All the extracts were rich in phenolic compounds and possess valuable antioxidant activities. Therefore, leaves and hull of *Pistacia vera* L. could be used as cheap natural antioxidant in different applications.

Introduction

Oxidative damage is a major human health problem that causes several diseases such as cancer and cardiovascular diseases. Antioxidants have a great role in biological systems; it prevents cell damage and reduces the risk of human illnesses [1]. Synthetic antioxidants obtained from fossil fuels have been widely used in industrial applications such as food, pharmaceutical and cosmetic, but there is evidence that some of these compounds have side effects on human health as well as on the environment [2]. To decrease these effects, renewable sources, especially plants, are being used to obtain natural bio-compounds, particularly phenolic compounds, as natural antioxidants [3].

*Pistacia vera* L. is a dioecious shrub that can reach heights of 3 to 10 meters [4]. It belongs to the Anacardiaceae family, it is probably native to central and western Asia, and it is widely distributed in the Mediterranean, especially in North Africa [4–6]. *Pistacia vera* L. is cultivated in different regions of Tunisia and it is important for the national economy. The most important pistachio producing regions are Gafsa, Sidi Bouzid, Sfax and Kasserine. The production of pistachio in the region of Gafsa was 1200 tons in 2019, the number of trees in this region was 2 million 114 thousand plants spread over an area of 18 thousand 875 ha [7]. Pistachio is used as a consumption of dried nuts or as an additive in the food industry [5, 8, 9]. The production of pistachios generates a large amount of potentially valuable waste such as leaves and husks. These products have been used as a valuable resource for several remedies [10]. Nowadays, several scientific works have confirmed the biological properties of the different parts of *Pistacia*, such as grains, husks, gum, oleoresin and leaves [8, 10–12]. In addition, it has been demonstrated that consumption of pistachios reduces the levels of the main risk factors for cardiovascular disease (CVD), prevents the appearance of cancer [13] and lowers LDL cholesterol [14].
Besides the above-mentioned human health benefits, other potential beneficial effects have also been studied. Several researchers have reported that pistachio shells possess antimicrobial, antimutagenic and antioxidant activity [15–17]. *Pistacia* gum has been used against certain diseases of the stomach and respiratory tract [12]. Additionally, the oleoresin from *Pistacia vera* demonstrated an antinociceptive and anti-inflammatory activities [8, 18]. In other studies, the extracts of *Pistacia vera* L. leaves (pistachio) have revealed an antiemetic effect in young chickens and a remarkable antioxidant effect [11, 12, 19]. Furthermore, the essential oils of *Pistacia vera* have been reported to exhibit antimicrobial and antioxidant activities [10].

In order to assess the suitability of leaves and hulls of *Pistachia vera* L. as a source of biorenewable compounds, a chemical characterization of these biomass has been carried out, as well as the analysis of their extractives. For this purpose, the total phenolic content (TPC), total flavonoid content (TFC) and antioxidant properties of the extracts were determined to assess the potential use of the extracts in different applications.

**Materials And Methods**

**Chemicals and raw material**

Ethanol and gallic acid were obtained from Scharlau. Sigma-Aldrich supplied sulfuric acid, trolox, catechin, 2,2-diphenyl-2-picrylhydrazyl hydrate (DPPH), 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ). All solvents and chemicals were used without further purification.

The fresh hull and leaves (male and female) of *Pistacia vera* L. were picked on August and October 2019, respectively. The collection was located in the region of Gafsa (Tunisia), a natural place far from pollution. After harvest, the collected raw materials were washed to remove debris and all kinds of dust, and then they were dried at room temperature for 15 days in the dark. Then, the leaves and hull were dried in an oven at 45 °C for 24 hours. The dry leaves and hull were ground to a particle size of less than 0.5 mm, which were stored in glass vials protected from light and moisture.

**Chemical composition of raw material**

Chemical composition of raw materials was performed by the determination of ash, extractives, acid soluble lignin, klason lignin and polysaccharides.

Moisture was evaluated according to the Technical Report of National Renewable Energy Laboratory (NREL) TP510-42621. 1 g of each sample (ML, FL and Hu) was heated at 105 ± 3 °C for 24 h, and the residue was weighed. The ash content was determined according to the NREL TP-510-42622. 1 g of sample was incinerated at 575 ± 25 °C overnight and the combustion residue was weighed and reported as ash content of the dry sample.
The extractive content was determined using sequential Soxhlet extraction of 5 g of each sample with ethanol and distilled water for 24 h for each solvent. After drying the solid residue at 105 °C, the solubilized extractives were determined and reported as a percentage of the original dry sample.

The determination of Klason lignin, acid soluble lignin and carbohydrates content were carried out according to the NREL TP-510-42618. The extractive free raw material was subjected to an acid hydrolysis with 72% sulfuric acid at 30 °C for 1 h, after, water was added to reduce the acid concentration to 4%, and the hydrolysis was completed at 121 °C for 1 h in the autoclave. The mixture was filtrated and the obtained solid residue was dried at 105 °C for 24 h, then weighted to be considered as Klason lignin and reported as a percentage of the original dry sample. Soluble lignin was quantified by measuring the absorbance of the obtained liquid phase at 240 nm using UV–vis spectroscopy (Jasco V630 UV-VIS spectrophotometer).

The polysaccharide content of the filtrate was estimated by High Performance Liquid Chromatography (HPLC) using a Jasco LC Net II/ADC chromatograph equipped with a refractive index detector and a column Aminex HPX-87H, 300 × 7.8 mm (Bio-Rad Laboratories, USA). The mobile phase was H₂SO₄ 0.005 M at a flow rate of 0.6 mL/min at 50 °C. Glucose, arabinose, xylose and galacturonic acid were the standard monosaccharides and their retention time were used to estimate the polysaccharide composition. The polysaccharides were reported as a percentage of the original dry mass.

Samples were analyzed by Pyrolysis-Gas chromatography–Mass spectrometry (Py-GC/MS) using a Pyroprobe Pyrolyzer (5150, CDS Analytical Inc, Oxford, PA) connected to an Agilent 6890 gas chromatograph coupled to an Agilent 5973 mass spectrometer (Agilent Technologies In., USA). Samples were degraded in an inert atmosphere. The pyrolysis was performed at 500 °C for 10 s (2 °C/ms). The GC oven program started at 50 °C and was held for 2 min then it was raised to 120 °C (10 °C/min) for 5 min and after that the temperature was raised to 280 °C (10 °C/min) for 8 min and finally raised to 300 °C (10 °C/min) for 10 min. The mass spectra were compared to the National Institute of Standards and Technology (NIST) mass spectra library.

**Extraction method**

The extraction was performed according to the method described by Sillero et al. [20], in a temperature-controlled ultrasonic bath (Elmasonic 570 H, Elma) using ethanol-water (50/50 (v/v)) mixture as solvent. Extracts (male leaves extract (MLE), female leaves extract (FLE) and hull extract (HuE)) were prepared using 4 grams of each dry material. The samples were placed in a glass bottle, and the used solid/liquid ratio was 1:10 (w/v). After 1 hour at 50 °C, the samples were filtrated under vacuum; the supernatant extracts were used to determine the amounts of total phenolic and flavonoid content, and to evaluate the antioxidant activity. The solid phase was dried at room temperature and then, the extraction yields were calculated gravimetrically and referenced to a 100 g of dried material. Each assay was performed in triplicate.

**Characterization of leaves and hull extracts**
Total phenolic content (TPC) of the extracts was determined according to the Folin–Ciocalteu method following the methodology described by Sillero et al. [20]. The absorbance was read at 760 nm using a Jasco V-630 spectrophotometer. Gallic acid was used as standard, and its calibration curve was determined with concentration ranging from 0 to 0.35 g/L. The equation of calibration curve was: \( y = 0.1348x - 0.007; R^2 = 0.9991 \). The total phenolic content was expressed in mg of gallic acid equivalent/g of dry extract (mg GAE/g DE).

Total flavonoid content (TFC) of each extract (MLE, FLE and HuE) was determined by the aluminium chloride colorimetric assay using the methodology detailed by Sillero et al. [20]. The absorbance was read at 510 nm, and catechin was used as a standard. Solutions of different catechin concentrations, from 0 to 0.824 g/L, were used to plot the calibration curve. The total flavonoid content was expressed as mg catechin equivalent/g of dry extract (mg CE/g DE). The catechin calibration curve equation was \( y = 1.1185x - 0.0141; R^2 = 0.9971 \). All experiments were performed in triplicate.

Fourier transform infrared (FTIR) spectroscopy was performed to identify the functional groups of ML, FL, Hu and their extracts. For that, a Perking Elmer Spectrum Two equipped with Universal Attenuated Total Reflectance accessory was used. The spectral range of 500 to 4000 cm\(^{-1}\) with a resolution of 4 cm\(^{-1}\) and 12 scans was used.

The molecular weight (Mw), number-average (Mn) and polydispersity index (Mw/Mn) of the extracts were determined by High Performance Size Exclusion Chromatography (HPSEC). The used chromatograph was a Jasco LC-NetII/ADC equipped with a RI-2031 Plus reflex index detector, Polar Gel-M guard (50 × 7.5 mm) and two Polar Gel-M columns in series (300 × 7.5 mm). Dimethylformamide with 0.1% of lithium bromide was used as mobile phase at a flow rate of 0.7 mL/min, and the volume injected was 20 µL. For calibration, polystyrene standards provided by Sigma Aldrich (70000–266 g/mol) were used.

**Antioxidant capacity of the extracts**

The determination of antioxidant activity of the extracts (MLE, FLE and HuE) was carried out using three methods. For all the methods trolox was used as standard and the results were expressed as mg of trolox equivalent (TE)/g of dry extract using a calibration curve with different trolox concentration solutions for each assay. The Jasco V630 UV-VIS spectrophotometer was used for all the tests.

The DPPH and ABTS assays were used to determine the rate of scavenging of free radicals, this assay is based on hydrogen atom transfer. The FRAP essay has been widely used to determine the ferric ion reducing power and it is based on electron transfer.

The DPPH radical scavenging assay was performed using the method described by Gullon et al. [21]. Briefly, 3 mL of DPPH solution (6 \(10^{-5}\) mol/L) was added to 0.3 mL of ethanolic solution of each extract (MLE, FLE and HuE). The decrease in absorbance was measured at 515 nm after 15 min of incubation at
room temperature. To plot the calibration curve of trolox, stock standard solutions in the range of 0 to 0.062 g/L were used. The equation of calibration curve of trolox was $y = -0.1304x + 0.0696; R^2 = 0.9983$.

The trolox equivalent antioxidant capacity (TEAC) was performed by the ABTS radical cation scavenging assay following the methodology described by Sillero et al. [22]. Briefly, 30 µL of each extract solution (MLE, FLE and HuE) was mixed with 3 mL of the ABTS solution. The absorbance was recorded at 734 nm after 6 min. Trolox solutions with different concentrations in the range of 0-0.712 g/L, were used in order to plot the calibration curve. The equation of calibration curve of trolox was $y = -0.9604x + 0.7174; R^2 = 0.9961$.

The ferric reducing antioxidant power (FRAP) assay was carried out using the method detailed by Sillero et al. [20]. This test was based on the reduction of the complex ferric Fe(III)–2,4,6-tripyridyltriazine (Fe(III)–TPTZ) to ferrous Fe(II)–TPTZ [21]. Briefly, 100 µL of each extract (MLE, FLE and HuE) was added to 3 mL of prepared reactive solution. The absorbance was measured at 593 nm after 6 min. The regression equation of calibration curve of trolox was $y = 0.3775x - 0.0137; R^2 = 0.9978$, determined using different trolox solutions with concentrations in the range of 0-0.54 g/L.

**Results And Discussion**

**Raw material characterization**

The male leaves (ML), female leaves (FL) and hulls (Hu) of *Pistacia vera* L. were characterized, the chemical composition of samples (ash, extractives, lignin, cellulose and also hemicellulose) were presented in Table 1.

|                          | ML       | FL       | Hu       |
|--------------------------|----------|----------|----------|
| Ash                      | $10.40 \pm 0.07$ | $8.89 \pm 0.02$ | $10.90 \pm 0.02$ |
| Total extractives        | $39.27 \pm 0.95$ | $43.17 \pm 0.91$ | $46.53 \pm 0.65$ |
| Water                    | $25.05 \pm 1.78$ | $32.18 \pm 0.68$ | $35.11 \pm 0.81$ |
| Ethanol                  | $13.93 \pm 0.65$ | $10.98 \pm 0.84$ | $11.93 \pm 0.16$ |
| Soluble lignin           | $6.55 \pm 0.17$ | $6.45 \pm 0.21$ | $5.26 \pm 0.09$ |
| Klason lignin            | $16.48 \pm 0.62$ | $14.51 \pm 0.12$ | $16.24 \pm 1.01$ |
| Cellulose                | $8.48 \pm 0.24$ | $9.02 \pm 0.33$ | $9.83 \pm 0.06$ |
| Hemicellulose            | $9.67 \pm 0.06$ | $10.28 \pm 0.15$ | $13.83 \pm 0.42$ |
In terms of the total ash content, FL has the lowest ash content 8%, while ML and Hu have similar value. Water and ethanol were used in order to determine the total extractive content in leaves and hull. All samples showed remarkable total extractives content; the highest value was the extractives from Hu (46%), followed by FL and ML. The extractive content is related to the solubility of the different compounds in the used extraction solvent. Water extractives were 35%, 32% and 25% for Hu, FL and ML, respectively. The amounts of ethanol extractives were lower than those measured for water. The highest ethanolic extractive content was for ML (13%), which means that this sample is rich in water-soluble compounds. The total lignin content was ranged from 20 to 22%. The Klason lignin content of the 3 samples varied between 14 and 16%, and the acid soluble lignin were between 5 and 6%. From the monosaccharides measured in the acid hydrolysis, the glucose was attributed to the cellulose content, while the hemicellulose content was provided the sum of galactose, arabinose and xylose [23]. The highest cellulose content was measured for FL and Hu, which had similar amounts, and the lowest content was determined in ML. The highest hemicelluloses content was detected for Hu of *Pistacia vera* L. (13.83%). In literature, different results were reported of cellulose (43.08% and 38.47%), hemicellulose (25.30% and 28.82%) and lignin (16.33% and 29.54%) in pistachio shells and in almond shells, respectively [24]. It should be noted that the chemical composition of the plants varies according to different conditions such as age, stage of growth and climatic conditions under which the plant grows [25].

In order to get a deeper knowledge on the chemical composition of ML, FL and Hu, pyrolysis coupled to GC/MS were carried out. The identified compounds are classified in the Table 2. The major identified component was 1-methyl-1H-Pyrrole, which is a nitrogen-containing compound; the highest content was detected in Hu (31.33%). Pyrrole and derivatives were formed form the pyrolysis of chlorophyll pigments [26], which explained the high N content in hull and leaves. Indole, styrene and phenol were also formed during pyrolysis from proteins and amino acids present in samples tissue [27]. In addition, furfural was identified as a product; this compound could be generated in the pyrolysis of residual carbohydrates [28]. Limonene, a monocyclic monoterpene, was detected with amounts of 15.28 and 17.46% for ML and FL, respectively; however, a low abundance of limonene was found for Hu (0.55%). This compound contributes to the flavors of the samples. Phenol, 2-methylphenol, 3-methylphenol, 4-ethylphenol, 2-methoxy-4-(1-propenyl)-phenol, 2,6-dimethoxy-4-(2-propenyl)-phenol, 2-methoxy-4-vinylphenol, 1,2-Benzenediol and 4-hydroxy-3,5-dimethoxybenzaldehyde detected in pyrolysis of the leaves and hull are compounds derived from the lignin. Leaves pyrolysis produced more lignin compounds than hull. Among the fatty acids released from the pyrolysis of samples, n-hexadecanoic and octadecenoic acids were the dominant fatty acids in ML. However, tetradecanoic acid was the most abundant in Hu. Alpha-tocopherol (vitamin E) and sitosterol were only detected in ML. The results suggested that leaves and hull of *Pistacia vera* L. are rich in natural bioactive compounds.
Table 2
Peaks assignments from chromatograms by PY-GC/MS with relative content/area (%), retention times (RT) and main fragments (m/z) of male and female leaves and hull of *Pistacia vera* L.

| Compound                                      | RT (min) | Main fragments (m/z) | Relative content/area (%) |
|-----------------------------------------------|----------|----------------------|---------------------------|
|                                               |          |                      | ML | FL | Hu |
| 2,5-dimethylfuran                             | 3.497    | 74/96/57             | 1.26 | 1.35 | 0.54 |
| 1-methyl-1H-Pyrrole                           | 3.999    | 81/80/53             | 17.80 | 14.75 | 31.33 |
| 3,3-diethylcyclopropene                       | 4.242    | 67/81/66             | — | 0.5 | — |
| Pyridine                                      | 4.282    | 87/73/77             | — | — | 1.56 |
| Toluene                                       | 4.496    | 91/92/57             | — | 5.12 | 3.95 |
| 5-Heptyn-3-ol                                 | 5.125    | 84/54/55             | — | 1 | — |
| 3-methyl-1H-Pyrrole                           | 6.361    | 91/92/65             | 0.50 | — | — |
| Furfural                                      | 6.096    | 96/95/82             | 2.08 | 5.12 | 3.95 |
| 1,3-dimethylbenzene                           | 7.303    | 91/106/86            | 3.01 | 1.00 | 1.85 |
| Styrene                                       | 8.14     | 104/107/103          | 1.31 | 0.53 | 1.24 |
| 5 Methylfurfural                              | 11.449   | 110/109/105          | 1.44 | 2.53 | 1.74 |
| Phenol                                        | 12.605   | 94/66/65             | 5.34 | 0.66 | 1.97 |
| 1-methyl-4-(1-methylethyl)-benzene            | 14.776   | 97/95/94             | 0.65 | 3.26 | 1.68 |
| Limonene                                      | 15.03    | 68/67/93             | 15.28 | 17.46 | 0.55 |
| Benzyl Alcohol                                | 15.434   | 97/67/68             | 0.68 | 0.63 | 0.75 |
| 2-methylphenol                                | 16.797   | 108/107/77           | 0.74 | 1.48 | 2.78 |
| 4-methylphenol                                | 18.131   | 107/108/77           | 2.78 | 0.60 | 0.55 |
| 2,4-dimethylphenol                            | 22.746   | 70/83/82             | 0.66 | — | 1.21 |
| 4-ethylphenol                                 | 23.999   | 107/122/121          | 2.39 | 1.77 | 2.88 |
| 1,2-Benzenediol                              | 26.679   | 110/64/63            | 4.00 | 5.76 | 1.56 |
| 4-ethyl-3-methylphenol                        | 28.66    | 121/91/69            | - | 0.73 | |
| 3-methoxy-1,2-benzenediol                     | 30.023   | 140/125/97           | 1.44 | 0.56 | 2.71 |
| Indole                                        | 32.033   | 117/90/89            | 1.79 | 0.64 | 1.50 |
| 2-Methoxy-4-vinylphenol                       | 33.367   | 150/135/77           | 1.53 | 1.00 | 1.42 |
| 2,6-dimethoxyphenol                           | 35.879   | 154/139/96           | 2.93 | 1.43 | 0.99 |
| Compound                                         | RT (min) | Main fragments (m/z) | Relative content/area (%) |
|-------------------------------------------------|----------|----------------------|---------------------------|
|                                                 |          | ML  | FL  | Hu  |
| Vanillin                                        | 38.969   | 151/152/81           | -  | 0.61 | -   |
| 2-methoxy-4-(1-propenyl)-phenol                 | 42.711   | 168/164/153          | 1.92 | 0.54 | 2.30 |
| 6,11-Dimethyl-2,6,10-dodecatrien-1-ol           | 43.704   | 69/81/95             | -  | 0.56 | -   |
| 1-(3,4-dimethoxyphenyl)-ethanone                | 47.007   | 180/165/137          | -  | 0.97 | -   |
| 4-hydroxy-3,5-dimethoxybenzaldehyde             | 49.006   | 178/99/156           | 0.86 | 17.46 | 2.29 |
| 2,6-dimethoxy-4-(2-propenyl)-phenol             | 49.665   | 194/91/179           | 1.65 | 0.83 | 2.33 |
| 1-(4-hydroxy-3,5-dimethoxyphenyl)-ethanone      | 50.184   | 181/196/153          | 2.69 | 0.86 | 0.93 |
| Tetradecanoic acid                              | 50.525   | 73/60/55             | 1.40 | 2.70 | 0.79 |
| Tridecanedial                                   | 51.478   | 68/95/82             | 1.59 | 2.15 | 4.48 |
| n-Hexadecanoic acid                             | 52.939   | 73/60/55             | 10.45 | 1.28 | 0.95 |
| Octadecenoic acid                               | 54.891   | 73/60/55             | 6.63 | 0.54 | 1.62 |
| Tridecylphenol                                  | 55.983   | 108/107/276          | -  | 0.70 | -   |
| Benzeneacetic acid,4-hydroxy-3-methoxy-, methyl ester | 56.555   | 137/151/138          | 0.93 | 0.49 | 1.07 |
| 4-Methoxy-4’,5’-methylene                       | 59.182   | 272/273/211          | 1.72 | -   | -   |
| Hexadecamethylheptasiloxane                     | 60.031   | 221/147/73           | -  | 0.62 | -   |
| α-Tocopherol                                    | 65.599   | 165/430/164          | 0.57 | -   | -   |
| Sitosterol                                      | 68.914   | 414/57/55            | 1.29 | -   | -   |

**Extraction yields and phenolic content**

Total phenolic content (TPC) and total flavonoid content (TFC) in leaves and hull extracts as well as the extraction yield are summarized in Table 3. The extraction yield of MLE, FLE and HuE were 28%, 29% and 41%, respectively (Table 3). It has been found that; ethanol–water is the most suitable solvent to obtain good extraction yields from plants [35]. Ozbek et al. [35] studied the effect of the ethanol–water ratio on the extraction yield from pistachio hull, reporting the highest yield (32.9%) at an ethanol–water ratio of 1/1 (v/v).
Table 3
Extraction yields, total phenolic content (TPC), total flavonoid content (TFC) of male and female leaves and hull extracts from *Pistacia vera* L. determined in this work, as well as different results reported by other authors

| UAE extract                  | Extraction yield (%) | TPC (mg GAE/g DE) | TFC (mg CE/g DE) |
|------------------------------|----------------------|-------------------|------------------|
| MLE                          | 28.87 ± 0.55         | 450.43 ± 20.33    | 373.18 ± 39.74   |
| FLE                          | 29.28 ± 0.45         | 533.41 ± 15.07    | 397.58 ± 10.18   |
| HuE                          | 41.64 ± 0.32         | 218.97 ± 15.36    | 119.75 ± 14.78   |
| *Ipomoea batatas* L. leaves [29] |                      | 112.98 ± 4.14     | 56.87 ± 5.69     |
| *Pistacia lentiscus* leaves [30] |                      | 147.68 ± 5.44     | 34.44 ± 0.93     |
| *Pistacia palaestina* leaves [31] |                      | 66.50 ± 2.20      | 20.30 ± 1.10     |
| *Pistacia vera* L. nuts [32]  |                      | 16.20 ± 0.40      | 7.2 ± 0.38       |
| *Ricinus communis* L. leaves [33] |                      | 623.7             | 213              |
| Ripe fruits of pistachio from Iran [34] |                      | 128.14 ± 0.30 -156.42 ± 0.50 |

For MLE and FLE the amounts of total phenolic content were significantly higher than that for hull, which was 218.9 mg GAE/g DE (Table 3). These values are higher than those reported for ripe fruits of five pistachio collected from Iran (128.1-156.4 mg GAE/g DE) [34], and also for those reported for other leaves such as leaves of *Pistacia lentiscus* [30], *Pistacia palaestina* [31], *Ricinus communis* [33] and *Ipomoea batatas* L. [29]. The TPC values obtained are lower than that provided by Ghnimi et al. [33] for *Ricinus communis* L. leaves (623.7 mg GAE/g DE). The highest flavonoid content was obtained for FLE (397 mg CE/g DE) followed by MLE and HuE. The measured TFC were higher than those reported in other studies for different leaves extracts collected in Table 3.

The content of TPC and TFC were higher in leaves than hull. It is because different parts of the same plant may synthesize and accumulate different amounts of phenolics content due to their differential gene expression. The richness in both phenolic and flavonoid compounds of the extracts of *Pistacia vera* L. leaves allows to consider this biomass as a potential source for their extraction.

**Infrared spectroscopy**

Figure 1 described the different bands and the corresponding wavenumbers in the region between 4000 and 500 cm$^{-1}$ of the 3 raw materials and their extracts. The spectral profiles of the extracts obtained by ultrasound-assisted extraction were very similar and contained the same principal chemical functional groups.

The broad band ranging from 3300 to 3200 cm$^{-1}$ correspond to the stretch vibration of OH groups of alcoholic and phenolic compounds [36]. The picks located in the region around 2988–2850 cm$^{-1}$ are
assigned for CH stretching including CH, CH₂ and CH₃ bending vibrations possibly derived from carbohydrates [37]. The bands around 1680–1740 cm⁻¹ were attributed to the presence of carbonyl and carboxylic acid groups vibrations [38]. The bands between 1620 and 1566 cm⁻¹ are the result of C–C stretching vibration of the aromatic ring and carboxylate group [38]. The peak in the region from 1600 to 1610 cm⁻¹ was assigned to conjugated C = C stretching vibrations of benzene ring of phenolic compounds [39]. The bands in the range of 1450 and 1400 cm⁻¹ were appeared in all samples and they were attributed to C = C aromatic bonds [39]. In addition, the peaks between 1386 and 1200 cm⁻¹ were attributed to C–O asymmetrical stretching vibration. The absorption peak around 1110 cm⁻¹ was attributed to aromatic C–H in-plane bending vibration and the band around 1065 cm⁻¹ was attributed to C–O stretching vibration [37, 38]. Bands between 930 and 750 cm⁻¹ were due to C–H out-of-plane bending vibration [37, 39].

**High performance size exclusion chromatography (HPSEC)**

HPSEC was used to evaluate the molecular weight distribution of obtained extracts from leaves and hull of *Pistacia vera* L. The molecular weight (Mw), the number-average (Mn) and the polydispersity index (Mw/Mn) are summarized in Table 4.

| Extract | Mn (g/mol) | Mw (g/mol) | Mw/Mn |
|---------|------------|------------|--------|
| MLE     | 621        | 7471       | 12.02  |
| FLE     | 695        | 9680       | 13.92  |
| HuE     | 372        | 501        | 1.34   |

The extracts consisted of a heterogeneous mixture of compounds with differentiated fractions. FL extract had the highest average Mw (9680 g/mol) and the highest average polydispersity index (13.92), followed by the MLE which had also high average Mw (7471 g/mol) and high average polydispersity index (12.02). The HuE had the lowest average Mw (501 g/mol) and the lowest average polydispersity index (1.34), which confirms that the compounds extracted from the hull are of low molecular weight. The leaves extracts were characterized by the presence of height molecular weight compounds with high polydispersity.

**Antioxidant activities**

The antioxidant capacities of the extracts of the three raw materials were studied in order to know their possible benefits. In this study, 3 chemical assays were used to study the antioxidant activity of leaves and hull extracts of *Pistacia vera* L. The results in Table 5 show that all different extracts had good antioxidant activity.
Table 5
The antioxidant activity of MLE, FLE and HuE (analyzed by the DPPH, ABTS and FRAP methods), as well as different results reported by other authors

|                  | DPPH       | ABTS       | FRAP       |
|------------------|------------|------------|------------|
| **MLE**          | 644.36 ± 31.20 | 1394.95 ± 5.49 | 808.24 ± 18.13 |
| **FLE**          | 704.53 ± 14.16 | 1334.35 ± 14.16 | 521.52 ± 4.11 |
| **HuE**          | 332.92 ± 23.89 | 427.06 ± 15.61 | 517.96 ± 26.62 |
| *Ceylon cinnamon* leaves [41] | 260.66 | 422.46 | 665.44 |
| *Ipomoea batatas* L. leaves [29] | - | - | 224 ± 2.20 |
| *Pistacia lentiscus* leaves [42] | 490 ± 13.10 | 336 ± 10 | 400 ± 14.10 |
| *Pistacia lentiscus* leaves [30] | 410.92 ± 45.87 | 327.85 ± 5.55 | - |
| *Pistacia palaestina* leaves [31] | 86 ± 2.75 | 13.275 ± 1.65 | - |
| *Pistacia vera* L. nuts [32] | 7.656 ± 0.19 | - | 33.125 ± 0.50 |

The values of trolox equivalent obtained with the DPPH assay were in the range 704 – 333 mg TE/g DE, and from 1394 to 427 mg TE/g DE with ABTS assay. The highest values for both assays were for FLE and MLE, respectively. The FLE and MLE, which are the extracts with the highest TPC where the most effective radical scavenger.

According to the results of FRAP assay, all the extracts achieved excellent results, between 517 and 808 mg TE/g DE. This test highlighted the high reducing power of MLE compared to FLE and HuE of *Pistacia vera* L. The reducing power of leaves and hull extracts is a good property once it can be interesting in the process of catalyzing LDL oxidation [40], among others. The antioxidant activities of leaves extracts from different species are reported in different studies (Table 5). Botsaris et al. [42] reported the values of DPPH, ABTS and FRAP for *Pistacia lentiscus* leaves aqueous extract. The measured values were 490, 336 and 400 mg TE/ g DE, respectively, which are lower than the values obtained in this work. The reducing ability of *Pistacia lentiscus* leaves is comparable with the result of FLE of this work and in the case of *Ipomoea batatas* L. leaves, studied by Zhang et al. [29], is below to those obtained in this study. Piluzza and Bullitta [30] reported high antioxidant activity for *Pistacia lentiscus* leaves compared to the values reported by Abu-Lafi et al. [31] for *Pistacia palaestina* leaves, which are far below to the values measured in this work. Something similar happen with the DPPH and FRAP values determined by Tsantili et al. [32] for pistachio nut extract, 7 and 33 mg TE/g DE, respectively. Regarding to other species, Abeysekera et al. [41] studied the antioxidant activity of the extract from *Ceylon cinnamon* leaves and showed values close to that determined for HuE in this work.
Pistacia vera L. leaves can be considered as an interesting source for valorization taking into consideration the antioxidant potential of their extracts. They could be used as a natural antioxidant instead of the synthetic ones in different applications such as food, cosmetic and pharmaceutical.

Conclusion

In this study, leaves and hull of Pistacia vera L. were characterized. This chemical characterization showed a high content on extractives and important amounts of phenolic compounds that explain their good antioxidant activities. Hu had the highest extractives content, FLE had the highest antioxidant potential given by DPPH and ABTS and MLE had the higher antioxidant potential given by FRAP. Therefore, both hull and leaves can be used as a source of natural antioxidant with potential applications in food, cosmetics and pharmaceutical industries. The use of this waste as a source of phytochemicals can provide new opportunities for the pistachio producers through the production of biobased antioxidant.

Further studies are needed to optimize the extraction technique and to identify the different components of extracts with antioxidant capacities.

Declarations

Acknowledgments

The authors greatly acknowledge the financial support of the Ministry of Higher Education and Scientific Research of Tunisia. L.S. would also like to thank the Department of Economic Development and Infrastructure of the Basque Government (scholarship of young researchers training).

Author contributions

ME and LS wrote the main manuscript text and done experimental part; LA, JL, YM interpreted the experimental data; All authors reviewed the manuscript.

Competing interests

The authors declare no competing interests.

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