Original Article

nutrition and health

Nutrition and Health
2025, Vol. 31(1) 321–330

DOI: 10.1177/02601060231183734
journals.sagepub.com/home/nah
Sage

Biological properties of extracts from the mixture of honey and berries of *Pistacia lentiscus*

Rabha Ayad[1], Nadia Amessis-Ouchemoukh[1], Amar Otmani[2], Nawel Guenaoui[2], Dyhia Sadou[1], Khodir Madani[1,3] and Salim Ouchemoukh[2]

Abstract

Background: Honey is a product of the hive elaborated by the bees of the species *Apis mellifera*. As well as *Pistacia lentiscus*, is a species widely used in traditional medicine that is part of a large family of *Anacardiaceae*. **Aims:** To determine the biological properties including the antioxidant activity of the mixture of the extract of *P. lentiscus* berries associated with honey at different concentrations (0.5%, 2%, 4%, 8% and 12%). **Methods:** Physicochemical parameters and phenolic compound amounts. The antioxidant activities (reducing power, FRAP, CUPRAC, TAC, DPPH, ABTS, and ferrozine) were also tested. **Results:** According to the physico-chemical parameters, the honey and the mixture analyzed are in conformity with the international standards. The results of the antioxidant assay gave a significant content of total phenolic compounds for the H/DP mixtures, the sample of honey alone analyzed exerts a weak antioxidant activity compared to the two mixtures made. **Conclusion:** The honey and *Pistacia* compound constitute an important source of antioxidants which intensifies very significantly the antioxidant activity of the blend.

Keywords

Honey, *Pistacia lentiscus*, physicochemical properties, antioxidants, antioxidant activities

Introduction

In Algeria, alternative medicine is still widely demanded by patients who are not satisfied with the treatments received and prefer natural and herbal remedies that seem to be more likely to be effective by their natural, non-harmful and inexpensive therapeutic effects (Hemlat et al., 2019).

Among the products of the hive, honey is elaborated by the bees of the *Apis mellifera* species from sweet substances (nectar and/or honeydew) that they take from various plants. Honey is not only a sweet food but a medicinal product as it is endowed with several biological activities such as: antimicrobial, antioxidant, anti-inflammatory and anticancer making it a first rank elixir (Oryan et al., 2016).

The uses of medicinal plants are attributed to their richness in secondary metabolites that constitute unique resources for pharmaceuticals, food additives and fine chemistry (Belhachat et al., 2018). *Pistacia lentiscus* L. belonging to the family *Anacardiaceae* is one of the most responded spontaneous plants in Algeria. This dioecious species can reach 3 m in height and grows in many Mediterranean countries (Pachi et al., 2020).

The traditional use of different parts of the plant for therapeutic purposes makes the mastic tree a medicinal plant. The inhabitants of rural areas use the infusion of its leaves to treat gastric ulcers, respiratory disorders and diabetes for its hypoglycemic effect. They also use decoctions of the aerial parts of mastic to treat toothache and throat infections (Sehaki et al., 2022).

The fruits are commonly eaten raw or roasted. In addition, the fruit oil represents a source of vegetable oils traditionally consumed in Tunisian and Algerian diets (Yosr et al., 2018; Milia et al., 2021). In North Africa, it is used to treat sore throats, locally to treat burns and wounds. The hydroalcoholic extracts of the berries were a rich

[1] Laboratoire de Biomathématique, Biochimie, Biophysique et Scientométrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, Bejaia, Algeria
[2] Laboratoire de Biochimie appliquée, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, Bejaia, Algeria
[3] Centre de recherche en technologie agro-alimentaires, Route de targua-ouzemour, Bejaia, Algeria

Corresponding author:
Rabha Ayad, Laboratoire de Biomathématique, Biochimie, Biophysique et Scientométrie, Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algeria.
Email: rabha.ayad@univ-bejaia.dz

source of phenolic compounds, including phenolic acids, flavanols, flavonols and anthocyanins (Elez Garofuli'c et al., 2020). In addition, changes in sterol and lipid composition with fruit ripening have been reported (Aissat et al., 2022).

The evaluation of the potential biological activities of *P. lentiscus* has been the subject of several scientific studies, which have demonstrated its therapeutic efficacy, thanks to its active compounds, including neuro-protective, antibacterial and antimicrobial, analgesic, antioxidant and antiproliferative activities in colon cancer in addition to its effectiveness against stomach ulcer. On the other hand, *in vivo* and *in vitro* studies have been carried out on *P. lentiscus* that reveal its anti-inflammatory and healing power (Brahmi et al., 2020; Mezni et al., 2020).

Our study consists in discovering the biological properties of *P. lentiscus* berries mixed with honey: determination of antioxidants and evaluation of the antioxidant activity of this mixture. The physicochemical characterization is also carried out in view of the formulation of a food with a more functional character and a more beneficial effect.

Materials and methods

Preparation of mixtures and methanolic extracts

The honey is the basic sample to which the berries of *Pistacia* are mixed with it. Samples were prepared by mixing a quantity of honey (H) with different concentrations of black berries of *P. lentiscus* (H/BB) and its powder of oilcake (H/DP) (0.5%, 2%, 4%, 8% and 12%) in order to obtain a quantity of 100 g.

After 15 days of preparation of the different mixtures, extractions were performed for each mixture. The principle of this extraction is based on the dissolution of the honey and the prepared mixtures (10 g) in the solvent (methanol/distilled water) (v/v). After shaking for 24 hours the solutions are centrifuged at 4500 rpm for 10 min and followed by filtration through a 0.45-µm filter, the methanolic solutions are kept cool in light-tight vials until use.

Physico-chemical analysis of honey and honey blend

The following physico-chemical parameters: humidity, Brix, pH and electrical conductivity, were analyzed according to the method reported by Bogdanov (1997) and Ayad et al. (2021).

Determination of antioxidants

Total phenolic compounds and flavonoids. Both tests were analyzed using the methods reported in our paper Ayad et al. (2021).

Flavonols

Flavonol contents in our extracts are determined according to the method reported by Adedapo et al. (2008). Flavonol contents are determined by referring to a calibration curve, obtained with quercetin (0.05 mg/ml) and are expressed in mg quercetin equivalents per 100 g of extract (mg QE/100 g) ($y = 15.16\ x + 0.009$; $R^2 = 0.999$).

Proanthocyanidins. The content of condensed tannins was determined according to the protocol described by Saad et al. (2012). The amount of condensed tannins was determined by the following formula:

$$C(\text{mg / g}) = \frac{(\text{AB} * \text{PM} * \text{DF} * 1000)}{(L * \varepsilon)} \quad (1)$$

AB: Absorbance of the extract.
PM: Molecular weight of ammonium iron sulfate (284.14 g/mol).
DF: Dilution factor.
L: Length of the cell (1 cm).
ε: Molar absorbance factor of ferric reagent (35,700 $\text{mol}^{-1}\ \text{cm}^{-1}$).

Determination of monomeric anthocyanins. The method used in this test is to Çam et al. (2009). The content of monomeric anthocyanins was expressed as mg cyanidin-3-glucoside equivalent per 100 g of extracts using the molar extinction coefficient (ϵ) of cyanidin-3-glucoside (Jackobek et al., 2007), according to the following formula:

$$\text{Monomeric anthocyanins(mg / L)} = \frac{(A * \text{MM} * \text{DF} * 10^3)}{\varepsilon * L} \quad (2)$$

Where:

A: $(A_{510\text{nm}} - A_{700\text{nm}})$ pH 1.0 - $(A_{510\text{nm}} - A_{700\text{nm}})$ pH 4.5;
MM (molar mass): 449.2 g/mol of cyanidin-3-glucoside;
DF: dilution factor;
3 (molar extinction coefficient): 26,900 L $\text{mole}^{-1}\ \text{cm}^{-1}$;
L (length of the cell): 1 cm.

Determination of carotenoids and L-ascorbic acid. Carotenoids were extracted according to the method of Sass-Kiss et al. (2005). Results are expressed as mg β-carotene equivalent per 100 g of extract (mg βCE/100 g) ($y = 119.73\ x$; $R^2 = 0.999$).

The vitamin C content was determined according to the method described by Klein and Perry (1982). Ascorbic acid (AA) concentrations are expressed as grams of AA per 100 g of extract (g AA/100 g) ($y = -5.132\ x + 0.701$; $R^2 = 0.994$).

Study of the antioxidant activity

The studied tests (reducing power; FRAP; CUPRAC; TAC; DPPH and ABTS) were analyzed using the methods that are already mentioned in our paper Ayad et al. (2021).

Metal chelation test (ferrozine). The chelating ability of the extracts was measured by the method of Le et al. (2007). The percentage inhibition is calculated according to the following formula:

$$\text{Antiradical activity}(\%) = \left[\frac{(\text{Abs}_{(t)} - \text{Abs}_{(e)})}{\text{Abs}_{(t)}}\right] \times 100 \quad (3)$$

$\text{Abs}_{(t)}$: Absorbance of the control.
$\text{Abs}_{(e)}$: Absorbance of the sample.

Statistical analysis

The results obtained are presented as the mean ± standard deviation of three replicates (n = 3). Normality and homogeneity of the data were checked for all variables by the Kolmogorov–Smirnov and Levene tests, respectively.

STATISTICA 7.1 software (Statsoft Co., Tulsa, OK, USA) was used to perform a one-factor analysis of variance classification (ANOVA) for the purpose of determining significant differences between samples at $p < 0.05$, and the LSD Fisher post hoc test was used for comparison of means. The Kruskal–Wallis ANOVA was applied to variables with inhomogeneous variances. Principal component analysis (PCA) and hierarchical ascending classification (HAC) were performed with STATISTICA software in order to reveal in a space of smaller dimensions while minimizing the loss of information, the similarities and differences between the different samples. In the hierarchical ascending classification, the Euclidean distance and Ward's method were used to evaluate the similarities between the samples.

Results and discussion

Physicochemical analysis of honey and mixtures

The results of the physicochemical analyses of the studied honey and the mixtures (H/BB and H/DP of *P. lentiscus*) at different concentrations (0.5%, 2%, 4%, 8% and 12%) are presented in the Table 1 below.

The value of the pH of the studied honey is 4.26, this value is increased when we add the berries of *P. lentiscus* before and after the delipidatiion, so the acidity of the honey is decreased but still remains in agreement with the international norms ($3.5 < \text{pH} < 5.5$), confirming the acid character of the honey (Table 1).

The electrical conductivity of honeys and mixtures (H/BB) and (H/DP) analyzed varies from 0.70 ± 0.00 mS/cm (H alone) to 0.98 ± 0.00 mS/cm (H/DP at 12%). The brix is calculated to estimate the carbohydrate content of the honey. The value indicated on the refractometer is 84.66% for the honey alone. A remarkable increase of the brix contents from the lowest to the highest concentration of the samples (H/BB) and (H/DP). This increase is explained by a richness of the DP and BB in sugar.

The water content of honey samples and mixtures (H/BB) and (H/DP) is between 9.93% (H/DP at 12%) and 13.21% (H alone) corresponding to refractive indices between 1.5121 and 1.5038. These results are lower than the maximum limit (20%) set by the European Union Council (2002). A remarkable decrease of the water content from the lowest to the highest concentration of the samples (H/BB) and (H/DP) indicating a good storage as well as ripeness degree.

The color results of the analyzed honeys ranged from 0.81 (H alone) to 2.85 (H/DP at 12%). A remarkable increase in color contents from the lowest to the highest concentration of the samples (H/BB) and (H/DP). This increase can be interpreted as a reliable indication of the presence of pigments with antioxidant activity such as carotenoids and flavonoids.

The protein results show that honey alone contains 10.14 mg EBSA/100 g, while the honey/*Pistacia* mixtures (BB and DP) at different concentrations (0.5%, 2%, 4%, 8% and 12%) present protein contents that vary from 33.77 to 143.96 mg E BSA/100 g for the H/BB mixture, and from 21.01 to 244.44 mg E BSA/100 g for the H/DP mixture.

The results of the sugar assay reveal that honey alone has a content of 76.81 ± 0.08 g GE/100 g, while honey/*Pistacia* mixtures (BB and DP) at different concentrations have high contents that vary from 85.61 to 115.48 g GE/100 g for H/BB mixtures, and from 77.89 to 105.88 g GE/100 g for H/DP mixtures.

According to the physico-chemical parameters (humidity, pH, electrical conductivity, color), the honey and the mixture analyzed are in conformity with the international standards.

Determination of antioxidants

The results of the determination of total phenolic compounds show that honey alone contains the lowest content with 72.70 mg GAE/100 g, while the honey/*Pistacia* mixtures (BB and DP) at different concentrations present high CPT contents that vary from 89.12 to 639.43 mg GAE /100 g for the H/BB mixture, and from 131.66 to 1087.81 mg GAE/100 g for the H/DP mixture (Figure 1 (a)). And all significantly different between them, the honey/DP mixtures contain more phenolic compounds than the honey/BB mixtures.

The CPT contents in our mixtures are higher compared to the values found by Tomczyk et al. (2020) for the mixture (rapeseed honey and black wall fruit (*M. bombycis* Kenmochi)) 59.7 ± 5.5 mg/100 g. This difference in total phenolic compounds concentrations can be explained by the addition of *Pistacia* seed powders which are known to be rich in these compounds.

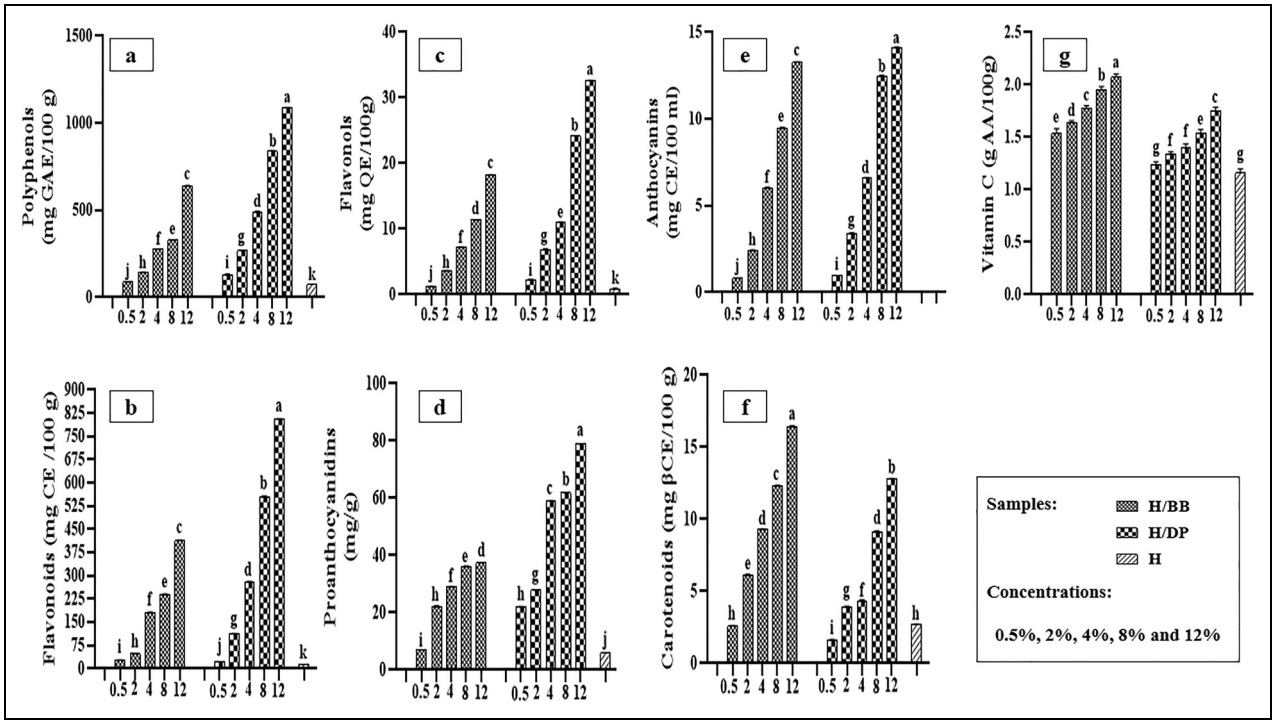

Figure 1. Antioxidant contents of the analyzed samples.
Different letter(s) indicate the values are significantly different (*$p < 0.05$). Vertical bars represent standard deviations.
GAE: Gallic Acid Equivalent; **CE:** Catechin Equivalent; **QE:** Quercetin Equivalent; **β-C E:** β-Carotene Equivalent; **AA:** Ascorbic Acid.
BB: Black Berries; **DP:** Dilipidated Powder.

According to Figure 1(b), the flavonoid contents of the different samples vary from 14.45 ± 0.18 to 804.85 ± 1.82 mg CE/100 g. Pure honey has the lowest flavonoid content, followed by the H/BB (0.5%), H/DP (0.5%) samples. The H/DP mixtures at 8% and 12% contain more flavonoids than the other mixtures and honey alone.

The results of the flavonol determination reveal that honey alone has the lowest content with 0.89 mg QE/100 g, while the honey/*Pistacia* mixtures (BB and DP) at different concentrations (0.5%, 2%, 4%, 8% and 12%) present higher flavonol contents that vary from 1.22 to 18.21 mg QE/100 g for the H/BB mixtures, and from 2.31 to 32.63 mg QE/100 g for the H/DP mixtures (Figure 1(c)). The H/DP blends at 8% and 12% contain more flavonols than the other blends and honey alone.

According to the histogram of Figure 1(d), the honey enriched in DP of *P. lentiscus* presents a significantly higher content of proanthocyanidins compared to the raw honey while this content increases with the increase of the powder concentration which means that the DP is the origin of this increase with values from 22.16 to 78.87 mg/g for H/DP and 5.90 mg/g for the honey alone. It is to be noted that all the H/BB samples present significant high contents compared to the honey alone with values from 7.21 to 37.41 mg/g but less low than the H/DP mixtures. This decrease can be explained by the presence of oil in BB.

From Figure 1(e), an increase in the amount of anthocyanins in the mixtures was observed while increasing the concentration of BB and DP from *P. lentiscus*. *P. lentiscus* berries remain a good source of anthocyanins, with values from 0.81 to 13.26 mg CE/L for honey/BB mixtures and from 0.96 to 14.09 mg CE/L for H/DP. While the lowest content is that of honey alone (0.03 mg CE/L). This difference in concentrations is confirmed by the addition of *Pistacia* seed powders which are known to be rich in these compounds.

The results shown in Figure 1(f) indicate that there is a significant difference between the analyzed samples (honey and the mixtures). A remarkable increase in carotenoid contents from the lowest to the highest concentration with ES values from 2.58 to 16.43 mg β-C/100 g for the H/BB samples and from 1.62 to 12.79 mg β-C/100 g for the H/DP mixtures. For the raw honey sample presents a content of 2.69 mg β-C/100 g.

While, for the H/DP mixtures, the carotenoid content is decreased, this is explained by the oil extraction, which studies showing the richness of *P. lentiscus* oil in carotenoids. According to Brahmi et al. (2020), the most abundant antioxidants in *P. lentiscus* oil are carotenoids 1480.0 ± 5.1 mg/kg.

According to Figure 1(g), the result of the determination of vitamin C shows that there is a significant increase of the contents for the mixtures H/BB with a value of 1.52 to 2.08 g AA/100 g, followed by the mixtures (1.24–1.75 g AA/100 g).

While the lowest content is the honey alone (1.16 g AA/100 g).

Evaluation of antioxidant activity

The results of the antioxidant activity evaluation are presented in Table 2.

The results obtained indicate that all samples have high antioxidant capacity. Moreover, the variation recorded between H, H/BB and H/DP is significant ($p < 0.05$). The highest capacity is observed for the H/DP mixtures, which presents a better efficiency for the studied tests followed by the H/BB mixtures. These results can be explained by the absence of reducing molecules in our extracts due to the presence of oil, which hinders the assays. However, the methanolic extract of honey alone reveals a significant capacity for all tests but low compared to the H/DP mixtures and the H/BB.

Moreover, the H/DP extract constitutes the good source of anthocyanins with a high content than the H/BB and honey alone, that is why present a high antioxidant capacity for all tests. Peña-Sanhueza et al. (2017) and Mohammedi, (2020) demonstrated a very remarkable content of anthocyanins, which are known for their high antioxidant capacity. The richness of the fruit in anthocyanins partly explains the high antiradical capacity of the fruit at this stage. The remarkable antioxidant properties of anthocyanins are the reason for their vital role in the prevention of neuronal, cardiovascular, diabetes and cancer diseases (Peña-Sanhueza et al., 2017).

According to several studies, there is a correlation between antioxidant properties and the content of bioactive compounds such as polyphenols, tannins, anthocyanins and flavonoids (Skrovankova et al., 2015; Nobre et al., 2018).

Antioxidant correlations and antioxidant activities

The correlation matrix between antioxidants and antioxidant activities was presented in Table 3. It revealed very highly significant relationships between antioxidants and antioxidant potential.

The polyphenol contents indicated very highly significant correlations ($p < 0.001$) with the different antioxidant activities ($r = 0.98$ with flavonoids and CUPRAC test; $r = 0.93$ with DPPH test; $r = 0.91$ with ABTS test; $r = 0.97$ with FRAP test and reducing power; $r = 0.86$ with ferrozine test).

Furthermore, the proportions of polyphenols and flavonoids in the analytical samples are in agreement with the results of Ouchemoukh (2012) and Canadovic et al. (2014) ($r = 0.83$; $r = 0.82$, respectively). As well as correlations noticed at $p < 0.001$ between flavonoids and DPPH test ($r = 0.87$) and ABTS test ($r = 0.85$). This is in agreement with the results of Ouchemoukh (2012) ($r = 0.83$).

The results showed a good correlation between carotenoid and vitamin C contents and antioxidant power, with the correlation coefficients shown in Table 3.

Several studies reported that phenolic compounds and flavonoids were directly responsible on the antioxidant activity of honey. According to Silici et al. (2010), the correlation coefficients obtained indicate that polyphenols contribute to the antioxidant activity of honey.

Multivariate analysis. The exploratory PCA analysis was carried out to study and compare the effect of the addition of black berries and cakes of the powder of the plant *P. lentiscus* on a sample of honey using different concentrations (0.5%, 2%, 4%, 8% and 12%) to determine the relationships between the variables studied with the help of a two-dimensional graphic representation. The principal components PC1 and PC2 which explain 90.78% of the total variation can be used for the appropriate explanation of the results obtained. Figure 2 shows that PC1 has the highest discriminating power with a value of 81.04% followed by PC2 which explains 9.74% of the data.

Figure 2(a) shows that the samples studied were divided into five groups. The first group containing the honey/DP mixture at 12% and the second group containing the honey/DP mixture at 8% followed by the third group containing the honey/BB mixture at 12% which is to the right of PC1 and therefore distinguished by their high content of bioactive compounds and the best antioxidant activity compared to the other studied mixtures and the honey alone. The fourth group, with the greatest number of samples of the honey mixtures, contained the mixtures closest to the average values of the different characteristics. The fifth group, on the opposite side of the graph (to the left of PC1) containing honey alone, presented the lowest levels of bioactive compounds and low antioxidant capacity. The results obtained also showed that the concentration has a significant effect on the levels of bioactive compounds and antioxidant capacity.

Figure 2(b) confirms the correlations obtained using the correlation matrix (Table 3) and shows strong correlations between the parameters studied. The correlation matrix and the PCA showed that the color of the honey is strongly correlated to its antioxidant content and has these antioxidant activities.

The hierarchical ascending classification (Figure 2(c)) revealed 5 clusters and these results confirm those obtained by the PCA. Cluster 1 was formed by honey/DP at 12% which represents the highest content of bioactive compounds and antioxidant capacity and the darkest color followed by cluster 2 and 3 which was presented by H/DP at 8% and H/BB at 12%, respectively. Cluster 4 with the largest number of samples (H/DP at 0.5%, 2% and 4%) and (H/BB at 0.5%, 2%, 4% and 8%) was characterized by average contents of bioactive compounds and antioxidant capacity followed by cluster 5 which was formed mainly by honey alone and showed lower contents and less dark color compared to other clusters.

Table 1. Results of physico-chemical parameters of analyzed honey and mixtures.

Samples	pH	EC (mS/cm)	Brix (%)	Refractive index (RI)	Humidity (%)	Color	Protein (mg EBSA/100 g)	Total sugar (g GE/100 g)
H	4.26 ± 0.12^{e}	0.70 ± 0.05^{k}	84.66 ± 0.14^{h}	1.5038 ± 0.06^{h}	13.21 ± 0.10^{a}	0.81 ± 0.05^{k}	10.14 ± 0.72^{k}	76.81 ± 0.08^{b}
H/BB 0.5%	4.62 ± 0.02^{d}	0.73 ± 0.03^{j}	85.17 ± 0.14^{g}	1.5043 ± 0.02^{h}	12.94 ± 0.02^{a}	1.55 ± 0.02^{j}	13.77 ± 0.70^{j}	85.61 ± 0.16^{h}
H/BB 2%	4.70 ± 0.01^{d}	0.77 ± 0.01^{h}	85.66 ± 0.10^{f}	1.5052 ± 0.04^{g}	12.65 ± 0.10^{b}	1.87 ± 0.02^{h}	37.92 ± 0.41^{h}	95.49 ± 0.14^{f}
H/BB 4%	4.82 ± 0.06^{c}	0.79 ± 0.03^{g}	85.92 ± 0.09^{e}	1.5063 ± 0.02^{f}	12.22 ± 0.08^{c}	2.13 ± 0.06^{g}	89.61 ± 0.42^{f}	98.86 ± 0.10^{c}
H/BB 8%	4.96 ± 0.01^{b}	0.89 ± 0.06^{d}	86.16 ± 0.40^{d}	1.5071 ± 0.01^{e}	11.89 ± 0.10^{d}	2.62 ± 0.01^{e}	126.33 ± 1.10^{d}	107.19 ± 0.08^{b}
H/BB 12%	5.03 ± 0.02^{b}	0.94 ± 0.02^{c}	86.75 ± 0.25^{c}	1.5085 ± 0.08^{d}	11.33 ± 0.04^{e}	2.78 ± 0.08^{c}	143.96 ± 0.41^{c}	115.48 ± 0.21^{a}
H/DP 0.5%	4.70 ± 0.05^{d}	0.74 ± 0.01^{i}	85.50 ± 0.25^{g}	1.5082 ± 0.05^{d}	11.45 ± 0.10^{e}	1.65 ± 0.05^{i}	21.01 ± 0.72^{i}	77.89 ± 0.16^{j}
H/DP 2%	4.98 ± 0.02^{b}	0.84 ± 0.05^{f}	86.58 ± 0.52^{d}	1.5093 ± 0.03^{c}	11.02 ± 0.05^{f}	2.43 ± 0.01^{f}	65.46 ± 1.10^{g}	84.16 ± 0.16^{i}
H/DP 4%	5.07 ± 0.02^{b}	0.87 ± 0.02^{e}	87.42 ± 0.14^{c}	1.5102 ± 0.01^{b}	10.65 ± 0.08^{g}	2.70 ± 0.03^{d}	99.27 ± 1.25^{e}	90.99 ± 0.14^{g}
H/DP 8%	5.29 ± 0.01^{a}	0.97 ± 0.04^{b}	87.67 ± 0.14^{b}	1.5113 ± 0.01^{a}	10.22 ± 0.09^{h}	2.83 ± 0.02^{b}	185.51 ± 0.72^{b}	100.97 ± 0.14^{e}
H/DP 12%	5.41 ± 0.01^{a}	0.98 ± 0.01^{a}	90.50 ± 0.25^{a}	1.5121 ± 0.04^{a}	9.93 ± 0.04^{i}	2.85 ± 0.04^{a}	244.44 ± 0.41^{a}	105.88 ± 0.10^{d}

Different letters indicate the values are significantly different ($^{*}p < 0.05$).
EBSA: equivalent of bovine serum albumin; GE: Glucose Equivalent.
H: honey ; BB: black berries; DP: dilipidated powder.

Table 2. Antioxidant activities of analyzed samples.

Samples	DPPH (%)	ABTS (%)	Ferrozine (%)	Pouvoir réducteur (mg GAE/100 g)	CUPRAC (mg TRE/100 g)	TAC (mg GAE/g)	FRAP (mg GAE/100 g)
H	26.89 ± 0.19^{k}	14.80 ± 0.13^{k}	32.92 ± 0.06^{j}	11.98 ± 0.02^{k}	21.83 ± 0.03^{j}	52.46 ± 0.23^{j}	15.77 ± 0.11^{j}
H/BB 0.5%	29.27 ± 0.19^{j}	30.84 ± 0.20^{j}	44.15 ± 0.11^{i}	14.08 ± 0.38^{j}	22.92 ± 1.13^{j}	58.57 ± 0.07^{i}	17.34 ± 0.14^{j}
H/BB 2%	31.56 ± 0.14^{i}	34.35 ± 0.20^{i}	48.01 ± 0.17^{h}	37.94 ± 0.21^{h}	79.24 ± 1.13^{h}	61.43 ± 0.13^{h}	113.70 ± 0.88^{h}
H/BB 4%	52.39 ± 0.12^{g}	43.47 ± 0.13^{h}	50.61 ± 0.11^{g}	60.14 ± 0.38^{f}	145.71 ± 0.56^{g}	67.13 ± 0.15^{g}	171.04 ± 0.33^{f}
H/BB 8%	72.07 ± 0.14^{d}	59.82 ± 0.15^{f}	54.55 ± 0.12^{f}	106.33 ± 0.43^{e}	250.49 ± 0.98^{e}	71.53 ± 0.15^{f}	258.11 ± 0.90^{e}
H/BB 12%	88.88 ± 0.14^{c}	80.75 ± 0.20^{d}	73.09 ± 0.13^{b}	139.70 ± 0.38^{c}	525.21 ± 1.50^{c}	76.99 ± 0.13^{d}	368.92 ± 0.57^{c}
H/DP 0.5%	35.84 ± 0.19^{h}	44.22 ± 0.20^{g}	50.87 ± 0.12^{g}	19.54 ± 0.43^{i}	63.74 ± 1.51^{i}	67.63 ± 0.13^{g}	73.61 ± 0.22^{i}
H/DP 2%	59.92 ± 0.12^{f}	65.15 ± 0.20^{e}	55.44 ± 0.17^{e}	51.89 ± 0.43^{g}	220.91 ± 1.51^{f}	74.61 ± 0.15^{e}	143.88 ± 0.44^{g}
H/DP 4%	69.99 ± 0.14^{e}	84.49 ± 0.20^{c}	57.89 ± 0.06^{d}	107.60 ± 0.21^{d}	324.82 ± 1.30^{d}	84.02 ± 0.13^{c}	304.35 ± 1.11^{d}
H/DP 8%	92.69 ± 0.19^{b}	93.02 ± 0.07^{b}	63.17 ± 0.17^{c}	173.71 ± 0.22^{b}	633.92 ± 1.30^{b}	87.09 ± 0.07^{b}	574.96 ± 0.55^{b}
H/DP 12%	95.16 ± 0.20^{a}	95.33 ± 0.13^{a}	76.96 ± 0.17^{a}	305.16 ± 0.21^{a}	988.86 ± 1.31^{a}	116.07 ± 0.07^{a}	695.62 ± 1.47^{a}

Values are mean $\pm$ standard deviation ($n = 3$). Different letters indicate significant differences ($p < 0.05$).
GAE: gallic acid equivalent; TRE: trolox equivalent.
H: honey; BB: black berries; DP: dilipidated powder.

Table 3. Correlation matrix between antioxidants and antioxidant activities.

	TPC	Flavonoids	DPPH	Proantho-cyanidins	Flavonols	ABTS	FRAP	CUPRAC	Ferrozine	TAC	PR	Carote-noids	Vitamin C	Antho-cyanins
TPC	1	***	***	***	***	***	***	***	***	***	***	***	**	***
Flavonoids	0.98	1	***	***	***	***	***	***	***	***	***	***	***	***
DPPH	0.93	0.87	1	***	***	***	***	***	***	***	***	***	**	***
Proanthocyanidins	0.89	0.82	0.87	1	***	***	***	***	***	***	***	***	**	***
Flavonols	0.98	0.97	0.89	0.90	1	***	***	***	***	***	***	***	***	***
ABTS	0.91	0.85	0.95	0.93	0.89	1	***	***	***	***	***	***	**	***
FRAP	0.97	0.94	0.90	0.94	0.99	0.92	1	***	***	***	***	***	***	***
CUPRAC	0.98	0.99	0.89	0.82	0.96	0.87	0.94	1	***	***	***	***	**	***
Ferrozine	0.86	0.83	0.87	0.85	0.88	0.93	0.88	0.85	1	***	***	***	***	***
TAC	0.89	0.85	0.85	0.89	0.89	0.88	0.89	0.87	0.87	1	***	***	*	***
PR	0.97	0.98	0.88	0.80	0.94	0.85	0.92	0.99	0.81	0.86	1	***	**	***
Carotenoids	0.78	0.81	0.80	0.57	0.75	0.71	0.71	0.81	0.76	0.67	0.84	1	***	***
Vitamin C	0.33	0.36	0.34	0.31	0.42	0.34	0.40	0.32	0.49	0.27	0.33	0.61	1	***
Anthocyanins	0.59	0.51	0.62	0.77	0.70	0.66	0.73	0.49	0.68	0.58	0.44	0.36	0.57	1

*Correlation different from zero ($p < 0.05$).
**Correlation different from zero ($p < 0.01$).
***Correlation different from zero ($p < 0.001$).

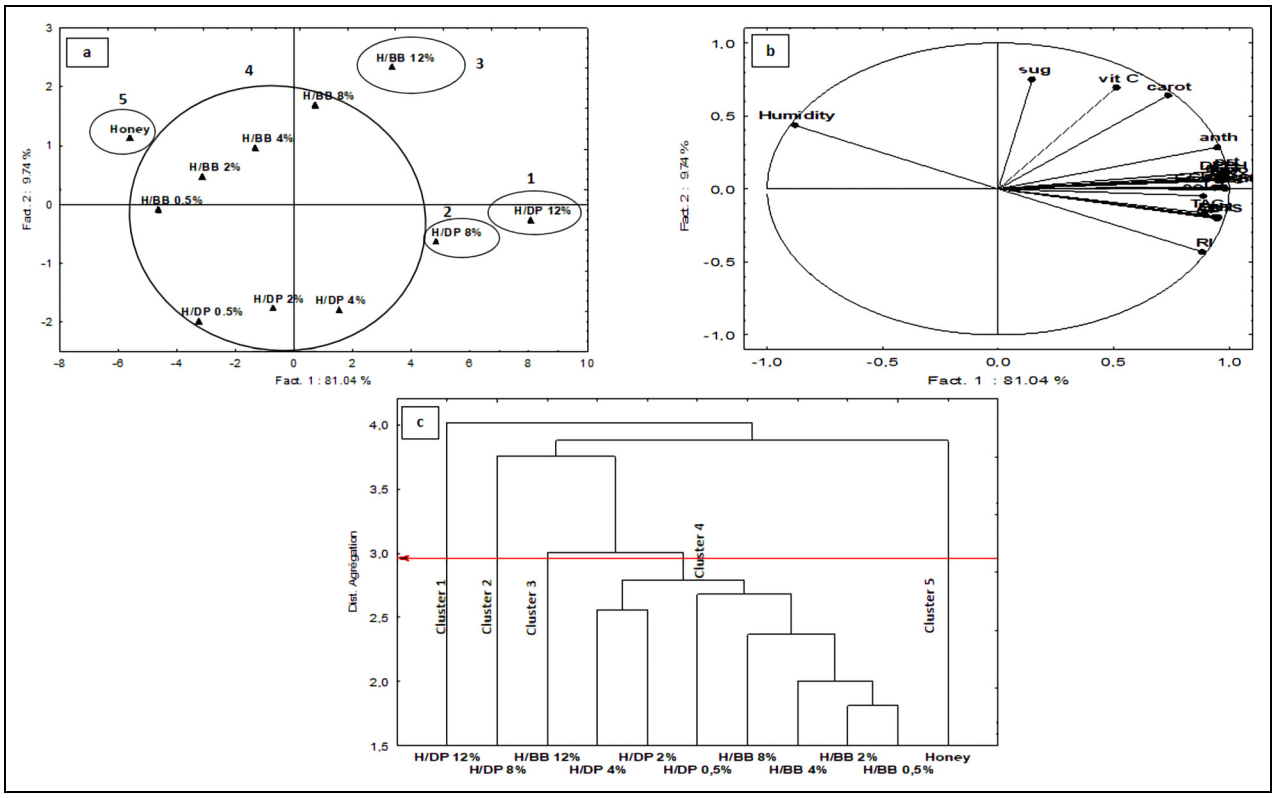

Figure 2. Biplot principal component analysis (PCA) of antioxidant compounds (phenolics, flavonoids, flavonols, proanthocyanidins, anthocyanins, carotenoids and vitamin C), antioxidant activity (DPPH and ABTS free radical scavenging, ferrozine, reducing power, CUPRAC, TAC and FRAP) and phsicochemical analyses. (a): projection of individuals on the factorial plane (1 × 2). (b): projection of the variables on the factorial plane (1 × 2).
DPPH: 2,2-diphinol-1-picrylhydrazyl; ABTS: 2-2-azinobis (3′-'ethylbenzothiazoline-6-sulfonic acid); RE: reducing power; CUPRAC: Cupric reducing antioxidant capacity; TAC: Total antioxidant capacity; FRAP: Ferric reducing-antioxidant power.

The results of the PCA and the hierarchical ascending classification show that the content of bioactive compounds and the antioxidant capacity of the mixtures of honey and honey alone depend on the species of the plant, on the nature of the powder and on the concentration used and notably on the color of these mixtures.

Conclusion

This work has allowed us, in the first place, to incorporate the berries of *P. lentiscus* at different concentrations in honey in order to formulate a food with a more functional character. On the other hand, to learn the analytical techniques passing from the extraction of polyphenols to the different methods of basic dosage in the study of the bioactive compounds contained in the honey and their mixture with the BB and the DP of *P. lentiscus*. This study allowed us to quantify the phenolic compounds using the appropriate universal techniques and the evaluation of the antioxidant capacity.

The results obtained show that honey is still a food source rich in active molecules that is used as an elixir since antiquity, moreover our honey meets the international standards of quality point of view; but our study whose objective is to add the powder of the berries of *P. lentiscus* to honey to evaluate the antioxidant activity of the mixture. Indeed, this compound allowed us to obtain a product very rich in total phenolic compounds with a more important fraction in comparison to flavonoids; the H/DP mixtures (12%) are richer than the H/BB mixtures, whose contents are 1087.81 mg GAE/100 g for phenolic compounds, 804.85 mg CE/100 g for flavonoids Likewise, the antioxidant activity determined by the reducing power test, FRAP, CUPRAC, TAC, DPPH, ABTS and ferrozine, appeared very significant, due to the abundant presence of total polyphenols.

In this study, we were able to demonstrate that honey and berries of *P. lentiscus* contains molecules that are considered as antioxidant agents could be used as ingredients in food industries and/or for therapeutic and pharmaceutical applications, knowing that antioxidants contribute in a very effective way to the prevention of diseases related to oxidative stress such as cancer, and cardiovascular diseases.

Author contributions

Conceptualization, RA and NAO; methodology, RA and NAO; software, RA, AO, NG and SO; validation, RA and NAO; formal analysis, RA; investigation, RA, DS and NAO; data

curation, RA; writing— original draft preparation, RA, SO, DS and NAO; writing—review and editing, RA, NG, SO and NAO; supervision, SO and NAO. All authors have read and agreed to the published version of the manuscript.

Availability of data and materials

No data available.

Consent for publication

Not applicable.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethics approval and consent to participate

Not applicable.

Funding

The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Ministry of Higher Education and Scientific Research, Algeria (Research Projects University Training PRFU project No: D01N01UN060120180005).

Human and animal rights

No human or animals were used for studies that are base of this research.

ORCID iD

Rabha Ayad https://orcid.org/0000-0002-5834-5242

References

Adedapo AA, Jimoh FO, Koduru S, et al. (2008) Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC Complementary and Alternative Medecine* 8(53): 1–8.

Aissat AK, Chaher-Bazizi N, Richard T, et al. (2022) Analysis of individual anthocyanins, flavanols, flavonols and other polyphenols in *Pistacia lentiscus* L. fruits during ripening. *Journal of Food Composition and Analysis* 106: 104286.

Ayad R, Amessis Ochemoukh N, Ouchemouk S, et al. (2021) Pollen profiles, physicochemical characteristics, and antioxidant activities of two honey samples from Jijel city (Algeria). *Fascicle VI—Food Technology* 45(2): 147–167.

Belhachat D, Mekimene L, belhachat M, et al. (2018) Application of response surface methodology to optimize the extraction of essential oil from ripe berries of *Pistacia lentiscus* using ultrasonic pretreatement. *Journal of Applied Research on Medicinal and Aromatic Plants* 9: 132–140.

Bogdanov S (1997) Nature and origin of the antibacterial substances in honey. *LWT-Food Science and Technology* 30(7): 748–753.

Brahmi F, Haddad S, Bouamara K, et al. (2020) Comparaison of chemical composition and biological activities of Algerian seed oils of *Pistacia lentiscus* L., *Opuntia ficus indica* (L.) mill. and *Argania spinosa* L. Skeels. *Industrial Crops and Products* 151: 112–456.

Çam M, Hısıl Y and Durmaz G (2009) Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chemistry* 112: 721–726.

Čanadanović-Brunet J, Ćetković G, Šaponjac VT, et al. (2014) Evaluation of phenolic content, antioxidant activity and sensory characteristics of Serbian honey-based product. *Industrial Crops and Products* 62: 1–7.

Elez Garofulíc I, Kruk V, Martíc A, et al. (2020) Evaluation of polyphenolic profile and antioxidant activity of *Pistacia lentiscus* L. leaves and fruit extract obtained by optimized microwave-assisted extraction. *Foods (Basel, Switzerland)* 9: 1556.

European Commission (2002) Directive 2001/110/EC of 20 December 2001 relating to honey. *Official Journal of the European Communities* 10: 47–52.

Hemlat N, Benarfa A, Beladel B, et al. (2019) Assessment of the contents of essential and potentially toxic elements in *Pistacia terebinthus* L. and *Pistacia lentiscus* L. by INAA technique. *Journal of Radioanalytical and Nuclear Chemistry* 332: 1127–1131.

Jackobek L, Šeruga M, Novak I, et al. (2007) Flavonols, phenolic acids and antioxidant activity of some red fruits. *Deutsche Lebensmittel Rundschau* 103: 369–378.

Klein BP and Perry AK (1982) Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *Journal of Food Science* 47: 941–945.

Le K, Chiu F and Ken NG (2007) Identification and quantification of antioxidants in *Fructus lycii*. *Food Chemistry* 105: 353–363.

Mezni F, Martine L, Khodja ML, et al. (2020) Identification and quantitation of tocopherols, carotinoids and triglycerides in edible *Pistacia lentiscus* oil from Tunisia. *Journal of Materials and Environmental Science* 11: 79–84.

Milia E, Bullitta SM, Mastandrea G, et al. (2021) Leaves and fruits preparations of *Pistacia lentiscus* L.: A review on the ethnopharmacological uses and implications in inflammation and infection. *Antibiotics* 10: 425.

Mohammedi Z (2020) Study of the evolution of the anti-free radical capacity of the fruit of *Arbutus unedo* L. at different stages of ripening. *Bulletin of the Royal Society of Sciences of Liege* 89: 130–146.

Nobre CB, Sousa SO, Camilo CJ, et al. (2018) Antioxidative effect and phytochemical profile of natural products from the fruits of "babaçu" (*Orbignia speciose*) and "buriti" (*Mauritia flexuosa*). *Food and Chemical Toxicology* 121: 423–429.

Oryan A, Alemzadeh E and Moshiri A (2016) Biological properties and therapeutic activities of honey in wound healing: A narrative review and meta-analysis. *Journal of Tissue Viability* 25(2): 98–118.

Ouchemoukh S (2012) Physicochemical Characterization, Pollen, Carbohydrate and Phenolic Profiles and Antioxidant Activities of Algerian Honeys. PhD thesis, University Abderrahmane Mira of Béjaia, Algeria 68–100.

Pachi VK, Mikropoulou EV, Gkiouvetidis P, et al. (2020) Traditional uses, phytochemistry and pharmacology of Chios mastic gum (*Pistacia lentiscus* var. Chia, *Anacardiaceae*): A review. *Journal of Ethnopharmacology* 254: 112485.

Peña-Sanhueza D, Inostroza-Blancheteau C, Ribera-Fonseca A, et al. (2017) Anthocyanins in berries and their potential use

in human health. Chapter 8. In: *Superfood and Functional Food – The Development of Superfoods and Their Roles as Medicine*. Temuco Catholic University: Intech Publisher, 20: 978-953-51-2941-7.

Saad H, Charrier-El Bouhtoury F, Pizzi A, et al. (2012) Characterization of pomegranate peels tannin extractives. *Industrial Crops and Products* 40: 242.

Sass-Kiss A, Kiss J, Milotay P, et al. (2005) Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Research International* 38: 1023–1029.

Sehaki C, Jullian N, Choque E, et al. (2022) Profiling of essential oils from the leaves of *Pistacia lentiscus* collected in the Algerian region of Tizi-Ouzou: Evidence of chemical variations associated with climatic contrasts between littoral and mountain samples. *Molecules* 27: 4148.

Silici S, Sagdic O and Ekici L (2010) Total phenolic content, antiradical, antioxidant and antimicrobial activities of Rhododendron honeys. *Food Chemistry* 121: 238–243.

Skrovankova S, Sumczynski D, Mlcek J, et al. (2015) Bioactive compounds and antioxidant activity in different types of berries. *International Journal of Molecular Sciences* 16(10): 24673–24706.

Tomczyk M, Zaguła G and Dżugan M (2020) A simple method of enrichment of honey powder with phytochemicals and its potential application in isotonic drink industry. *LWT-Food Science and Technology* 125: 109204.

Yosr Z, Imen BHY, Rym J, et al. (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: 151–159.