RESEARCH ARTICLE

Antioxidant activity and enzyme inhibitory potential of *Euphorbia resinifera* and *E. officinarum* honeys from Morocco and plant aqueous extracts

Oumaima Boutoub[1] · Soukaina El-Guendouz[2] · Letícia M. Estevinho[3] · Vanessa B. Paula[3] · Smail Aazza[4] ·
Lahsen El Ghadraoui[1] · Brígida Rodrigues[5] · Sara Raposo[6] · Jorge Carlier[7] · Maria C. Costa[8] · Maria G. Miguel[2]

Abstract

Natural products may be applied in a wide range of domains, from agriculture to food and pharmaceutical industries. In this study, the antioxidant properties and the capacity to inhibit some enzymatic activities of *Euphorbia resinifera* and *Euphorbia officinarum* aqueous extracts and honeys were assessed. The physicochemical characteristics were also evaluated. Higher amounts of iron, copper and aluminium were detected in *E. officinarum* honey, which may indicate environmental pollution around the beehives or inadequate storage of honey samples. This honey sample showed higher amounts of total phenols and better capacity for scavenging superoxide anion free radicals and DPPH free radicals as compared with *E. resinifera* honey, but poorer capacity for inhibiting lipoxygenase, acetylcholinesterase, tyrosinase and xanthine oxidase. The ratio plant mass:solvent volume (1:100) and extraction time (1 - 2 h) were associated with higher total phenols and better antioxidant activities and lipoxygenase, acetylcholinesterase and tyrosinase inhibitory activities, regardless of the plant species. The aqueous extracts had systematically higher in vitro activities than the respective honey samples.

Keywords Antioxidant · Acetylcholinesterase · Lipoxygenase · Tyrosinase · Xanthine oxidase · Natural products

Introduction

Euphorbia officinarum L. and *E. resinifera* O. Berg are dicotyledonous plants of the Euphorbiaceae family (Bruyns et al. 2011). This family contains around 300 genera of plants (Ul-Haqa et al. 2012) being one of the largest and most cosmopolitan families in sub-branching angiosperms (Vasas et al. 2012; Chis et al. 2012). The floristic diversity in Morocco (Chakir et al., 2016) allowed the use of a wide variety of plants for therapeutic and medicinal purposes since hundreds of years. *Euphorbia resinifera* and *E. officinarum* are one of the oldest "drugs" in the Western medical tradition,

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✉ Maria G. Miguel
    mgmiguel@ualg.pt

1   Laboratory of Functional Ecology and Environment, Faculty of
    Science and Technology, University Sidi Mohamed Ben Abdallah,
    BP 2202, 30 000 Fez, Morocco

2   Mediterranean Institute for Agriculture, Environment and
    Development, Faculty of Science and Technology, University of
    Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

3   CIMO-Centro de Investigação de Montanha, Instituto Politécnico de
    Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

4   Laboratory of Phytochemistry, National Agency of Medicinal and
    Aromatic Plants (ANPMA), BP 159, Principal,
    34000 Taounate, Morocco

5   Centre for Marine and Environmental Research (CIMA),
    Universidade do Algarve, Campus de Gambelas,
    8005-139 Faro, Portugal

6   Centre for Marine and Environmental Research (CIMA), Faculdade
    de Ciências e Tecnologia, Universidade do Algarve, Campus de
    Gambelas, 8005-139 Faro, Portugal

7   Centre of Marine Sciences (CCMAR), University of the Algarve,
    Gambelas Campus, 8005-139 Faro, Portugal

8   Centre of Marine Sciences (CCMAR), Faculdade de Ciências e
    Tecnologia, University of the Algarve, Gambelas Campus,
    8005-139 Faro, Portugal

being much used by Moroccan herbalists and therapists (Farah et al. 2014). "Zaggoume", the Arabian name for *E. resinifera*, is an endemic species of Morocco mainly distributed in the middle of the country, in Azilal and Beni Mellal regions (Middle Atlas), with some scattered populations in the High Atlas and Anti-Atlas Mountains (Moujanni et al. 2018). "Daghmouss", the Arabian name for *E. officinarum*, is an endemic species of North Africa; in Morocco, this plant is distributed from the north of the Souss River until the Western Sahara reaching the region of Zemmour (Peltier and Msanda 1995).

Morocco is a favourable territory for beekeeping and honey production owing to its floral resources and climate (Chakir et al. 2016). The number of hives and beekeepers is estimated at 375,000 and 35,000, respectively. The total annual honey production is estimated at 3500 tonnes (Bettara et al. 2015). The *Euphorbia* honey is considered the most precious by the consumer (Bettara et al. 2015).

To date, most studies on *Euphorbia* species are focused on its latex (Kabbaj et al. 2012; Bruyns et al. 2011; Mwine and Van Damme 2011; Mazoira et al. 2011; Daoubi et al. 2007), with little emphasis on the other parts of the plant. The main goal of the present study was to compare some in vitro biological properties (inhibition of xanthine oxidase, lipoxygenase, acetylcholinesterase and tyrosinase enzymes' activities as well as antioxidant activities) of the aqueous extracts of the aerial part of *E. resinifera* and *E. officinarum* and of those of honey samples from the same monofloral origin. The physicochemical characterization of the two monofloral honey types was also studied.

Material and methods

Honey samples

Monofloral honey samples from *Euphorbia resinifera* and *Euphorbia officinarum* were acquired from the beekeepers in Morocco and kept under ambient temperature in the shelter of light. Table 1 depicts the coordinates where honey samples were collected as well as their melissopalynological profiles.

Plant samples

The aerial part of the plants (*E. resinifera* O. Berg and *E. officinarum* L.) was collected in July 2018 directly from the fields of Beni Mellal and Tiznit, Morocco (Fig. 1). Dried plant material from both species was deposited as authenticated vouchers in the Herbarium of the Universidade do Algarve (acronym ALGU), with the accession numbers 15745/ALGU and 15746/ALGU, respectively.

Extract preparation

Plant material was well dried at room temperature, in the dark and afterwards 1 g from the aerial parts of each species was extracted with 20 mL (50 mg/mL), 50 mL (20 mg/mL) and 100 mL (10 mg/mL) of distilled water (w/v) by decoction. For each volume of water used, three decoction times were assayed (30 min, 1 h and 2 h). Each sample was centrifuged at 5000 rpm, for 15 min. The supernatant was recovered and kept in – 20 °C until further analysis.

Physicochemical analysis of honey

The parameters pH, free acidity, lactonic acidity, total acidity, ash content, electrical conductivity, moisture, proline content, diastase activity, hydroxymethylfurfural (HMF) and reducing sugars were determined according to methodologies previously described in detail (Bogdanov 2009).

Melissopalynological analysis

The analysis of the honey samples' pollen qualitative and quantitative spectrum was performed according to the International Commission for Bee Botany (ICBB), as previously described (Louveaux et al. 1978). Pollen identification and count were carried out using a light microscope (Leitz Messtechnik GmbH; Wetzlar, Germany) with 400× and 1000× objectives.

Estimation of honey colour

The colour was determined by measuring the absorbance of the honey solution (1 g in 2 mL of distilled water) at 635 nm (A_{635}), using a UV-Vis spectrophotometer (Ultrospec™ 1100 pro UV/visible spectrophotometer). The mm Pfund values of honey samples were obtained using the following algorithm: mm Pfund = $- 38.7 + 371.39 \times A_{635}$ (Aazza et al. 2017)

Elemental analysis

Nine elements were quantified (Fe, Zn, Mn, Cu, Al, Ca, K, Mg, Na) according to the method previously detailed (Terrab et al. 2004). Ca, Mg, Mn, Zn, Cu and Fe were measured by flame atomic absorption spectroscopy (novAA 350, Analytik Jena, Germany), while, Na, K and Al by microwave plasma atomic emission spectroscopy (4200 MP-AES, Agilent, USA).

Carbohydrate content

For carbohydrate determination, the method previously described by Veena et al. (2018) with few modifications was followed. In brief, 5 g of honey was dissolved in water and

Table 1 Places of collection, year of production and the most predominant pollen of two *Euphorbia* honey samples from Morocco

Honey type	Pollen species (percentages, %)	Production region	Coordinates	Production year
Euphorbia resinifera	*E. resinifera* 48.7 ± 1.1	Beni Mellal-Khénifra	32° 20′ 22″ N, 6° 21′ 39″ W	2018
	Caesalpinia pulcherrima 21.8 ± 1.3			
	Malvus domestica 10.2 ± 0.4			
	Cistus crepis 7.9 ± 0.9			
	Populus nigra 4.0 ± 0.3			
	Genista hirsuta 2.9 ± 0.2			
	Populus alba 1.9 ± 0.2			
	Elix aquifolium 2.6 ± 0.3			
Euphorbia officinarum	*E. officinarum* 52.1 ± 1.6	Tiznit-Souss-Massa	29° 42′ 00″ N, 9° 43′ 37″ W	2018
	Caesalpinia pulcherrima 11.8 ± 0.7			
	Arbutus unedo 6.1 ± 1.2			
	Populus alba 5.8 ± 0.8			
	Pinus pinaster 5.0 ± 0.2			
	Eucalyptus globulus 3.3 ± 0.6			
	Malvus domestica 3.0 ± 0.2			
	Thymus lotocephalus 2.4 ± 0.3			
	Quercus suber 2.0 ± 0.1			
	Eucalyptus cinereae 1.9 ± 0.2			
	Populus nigra 1.8 ± 0.3			
	Caesalpinia spinosa 1.7 ± 0.1			
	Cistus albidus 1.7 ± 0.2			
	Trifolium arvense 1.5 ± 0.3			

acetonitrile (75:25, v/v) using a 100-mL volumetric flask. The solution was filtered (0.45 μm) and stored at 4 °C until analysis. The quantification was done through high-performance liquid chromatography, using a chromatograph (Hitachi LaChrom Elite HPLC, Japan) equipped with a refractive index detector (Hitachi L-2490, Japan). A Purospher STAR NH$_2$ (5 μm particle size) (Merck, Germany) column was used with an isocratic elution using as mobile phase acetonitrile and water (85:15, v/v) at room temperature. Monosaccharides and disaccharides were analyzed at a flow rate of 1 and 1.3 mL/min, respectively. Injection volume was set at 20 μL. The quantification method of every carbohydrate was made using standard solutions at different concentrations (0.5–50 mg/mL), which were injected in the same conditions of honey samples. A calibration curve was done (concentration versus peak area) for the quantification of carbohydrates present in honey samples.

Total phenol content

The total polyphenol content in honey and plant samples was determined as stated by El-Guendouz et al. (2016). The reducing sugars interfere with Folin-Ciocalteu's phenol reagent; therefore, a solution with the same concentrations of glucose and fructose detected in honeys was used as control. The difference of absorbances observed was used to determine the total polyphenol content which was expressed as mg gallic acid equivalents (GAE) per g for plant extract and GAE/100 g for honey.

Antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity

DPPH free radical scavenging activity was assessed as described by El-Guendouz et al. (2018) in which 25 μL of the plant extracts or 200 μL of honey samples was added to 250 μL of DPPH solution (63.4 μM) and incubated for 30 min at room temperature; the absorbance was measured at 517 nm. The result was calculated using the following formula: Inhibition = [(A_0−A_1)/A_0) ×100], with A_0 is the absorbance of the control and for A_1 is the absorbance of the sample. The sample concentration providing 50% inhibition (IC_{50}) was achieved by plotting the inhibition percentage against samples' concentrations.
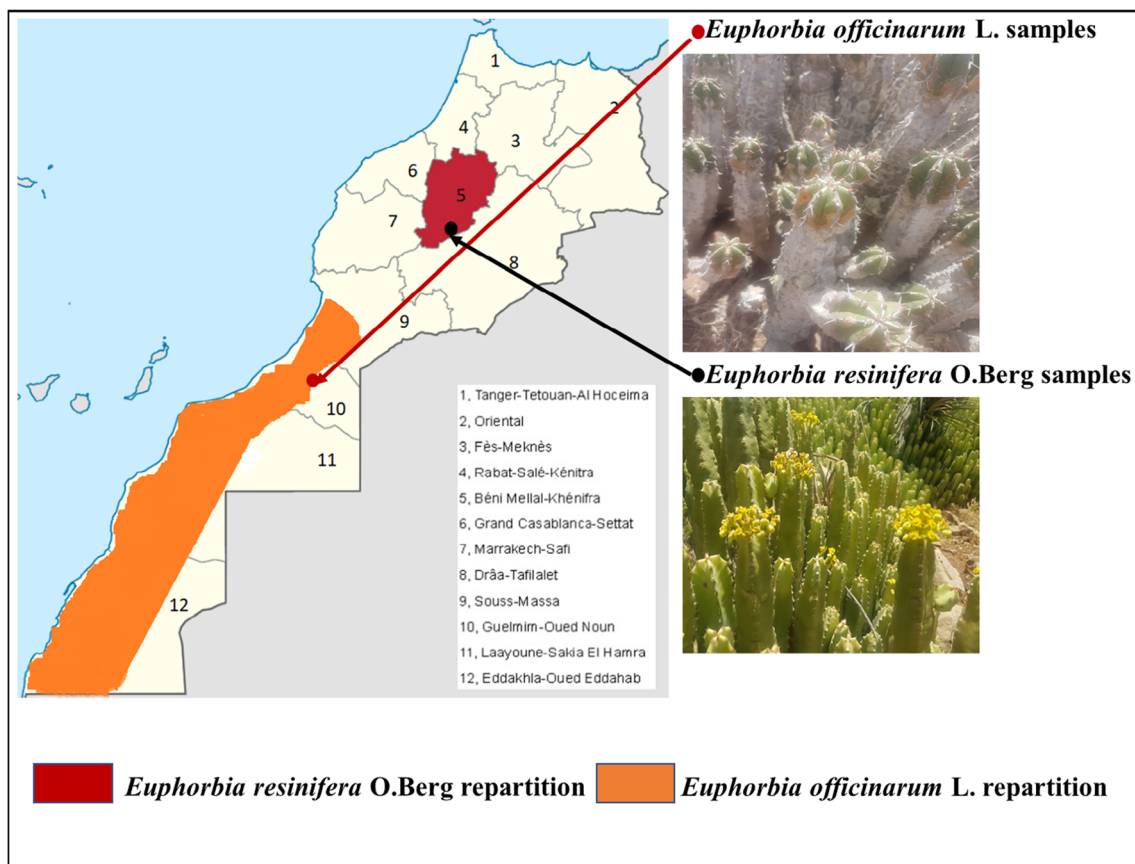
Fig. 1 Range of the 2 plants (*Euphorbia resinifera* O. Berg, *Euphorbia officinarum* L.) as well as the distribution of the samples studied in Morocco

Butylated hydroxytoluene (BHT) was used as standard (0.03–1 mg/mL).

Nitric oxide scavenging activity

The nitric oxide (NO) scavenging activity was carried out according to the manufacturer's instructions (Griess reagent kit 2003). The result was calculated using the following formula: Inhibition = $[(A_0-A_1)/A_0) \times 100]$, with A_0 is the absorbance of the control and for A_1 is the absorbance of the sample. The concentration of the sample that allows 50% inhibition (IC_{50}) was obtained by drawing the percentage of inhibition curve. Curcumin was used as standard (0.03–1 mg/mL).

Scavenging ability of superoxide anion radical

The scavenging ability of superoxide anion radical was assayed as reported by El-Guendouz et al. (2018). The result was calculated using the following formula: Inhibition = $[(A_0-A_1)/A_0) \times 100]$, with A_0 is the absorbance of the control and for A_1 is the absorbance of the sample and the IC_{50} value was determined as reported previously. Ascorbic acid was used as standard (0.03–1 mg/mL).

Enzymatic activities

Inhibition of acetylcholinesterase

The acetylcholinesterase inhibition was carried out with few modifications as reported by El-Guendouz et al. (2016). About 50 μL of plant extract or 300 μL of honey solution was used in the assay. The percentage of inhibition of acetylcholinesterase activity was determined and the IC_{50} value was calculated. Galantamine was used as standard (0.03–1 mg/mL).

Inhibition of lipoxygenase

The lipoxygenase assay was used as an indicator of anti-inflammatory and antioxidant activity (Silva et al. 2016). The inhibition action of honey solution and plant extract was determined as previously reported (El-Guendouz et al. 2016), with some modifications. In short, 100 μL of plant extract or 150 μL of the honey solution was used in the assays. The inhibitory effect of the test was calculated by comparison with negative control: Inhibition % = $[(A_0-A_1) / A_0] \times 100$, with A_0 is the absorbance of the blank sample and A_1 is the absorbance of the sample. The results were expressed as IC_{50} value. Nordihydroguaiaretic acid (NDGA) was used as standard (0.03–1 mg/mL).

Inhibition of tyrosinase

The tyrosinase inhibitory activity was determined based on the protocol proposed by El-Guendouz et al. (2016), with slight modifications. The total assay mixture consisted on adding 50 μL of honey solution or plant extract, mixed with 170 μL of phosphate buffer (50 mM; pH = 6.5) and 20 μL of tyrosinase enzyme (100 U/mL); the mixture was incubated for 40 min. After this period, 60 μL of L-Dopa (5 mM) was added. The reading was done at 492 nm, the inhibition percentage of the enzyme was calculated, and the IC_{50} values were compared. Kojic acid was used as standard (0.006–1 mg/mL).

Inhibition of xanthine oxidase

The inhibitory activity of plant extracts and honey solutions on xanthine oxidase was determined as described by El-Guendouz et al. (2016), with minor modifications. For that, 50 μL of plant extract or 150 μL of honey solution was used in the assays. The percentage of inhibition of xanthine activity was calculated and the IC_{50} was determined.

Statistical Analysis

Statistical analysis was performed with the Software Package of Statistics for the Social Sciences (SPSS) 25.0 (SPSS Inc., Chicago, IL, USA). Statistical comparisons were made with a one-way analysis of variance followed by multiple Tukey's comparisons. The level of significance was set at 5%. Correlations between phenol and antioxidant activity levels and enzyme inhibitory activities were obtained by the Spearman (r) correlation coefficient at a 95% level of significance. To run PCA (Principal Component Analysis), the PAST statistics version 4 software (Øyvind Hammer, Natural History Museum, University of Oslo) was used. Prior to each PCA running, the data was normalized by subtracting the mean value and dividing by the standard deviation of each of the given values. The same procedure and program were used to build the matrix plot. The dendrogram was obtained based on correlation and using Ward's method.

Results

Honey samples

Melissopalynological and general physicochemical properties

The predominant pollen grain analysis allowed to classify the samples as being monofloral honeys from *E. resinifera* and *E. officinarum* honeys (Table 1). In the case of *E. resinifera* honey, a secondary pollen type was identified: *Caesalpinia pulcherrima* (L.) Sw that exceeded 20%. In the

E. officinarum honey, the difference between the percentage of the predominant pollen grains of *E. officinarum* (52.1%) and the secondary pollen grains of *Caesalpinia pulcherrima* (11.8%) was higher (Table 1).

In this study, pH values for both monofloral honey types were 4.1 (Table 2). The free acidity values found in the present work did not exceed 10.6 meq/kg for both honey types (Table 2), and were slightly lower than 18.2 meq/kg. Regarding lactonic acidity, *E. officinarum* honey exhibited higher value of 10.96 meq/kg, compared with that of *E. resinifera* honey (7.68 meq/kg). Finally, the values recorded for the total acidity were as follows: 17.76 meq/kg for *E. resinifera* honey and 21.60 meq/kg for *E. officinarum* honey (Table 2).

The values of moisture were 18.69% for *E. resinifera* and 19.00% for *E. officinarum* (Table 2). The values of electrical conductivity obtained in the present study were 379 μS/cm for *E. resinifera* and 342 μS/cm for *E. officinarum* (Table 2). The values of diastase activity were above 8.0 Shade units/g (Table 2). In this study, the amount of proline detected in *E. officinarum* honey was 730 mg/kg while *E. resinifera* honey presented greater amount of 954 mg/kg (Table 2). The two studied honey samples presented the same ash content (0.14%) (Table 2). *E. officinarum* honey had 89.4 mg/kg (Table 2); the value surpasses the limit permitted by the European regulations. Regarding *E. resinifera* honey, the value found was 2.30 mg/kg (< 40 mg/kg). Concerning honey colour, both samples had a mm Pfund > 114, corresponding to a dark amber colour (colour Pfund scale = 408 mm for *E. resinifera* and 295 mm for *E. officinarum*) (Table 2).

For *E. resinifera*, the percentage of reducing sugar was 66.7% and for *E. officinarum* was 61.7%; therefore, they are within the range of the quality standards (Table 2). The results of the sugars profiling are summarized in Table 2. According to the results depicted in Table 2, the *E. resinifera* honey had higher amount of fructose (37.0 ± 0.8 g/100 g) when compared with *E. officinarum* honey (34.9 ± 0.1 g/100 g). Concerning glucose in both honey samples, the amounts were 30.2 ± 30 and 34.1 ± 3.8 g/100 g for *E. resinifera* and *E. officinarum* honeys, respectively. In honey samples, it was also possible to find sucrose, trehalose, maltose and turanose (Table 2); nevertheless, the levels were different depending on the honey type. *E. officinarum* honey had higher amounts of trehalose, maltose and turanose but lower amounts of sucrose than *E. resinifera* honey.

The analysis of the mineral elements in both honey samples revealed the predominance of potassium with a value of 394 mg/kg in *E. officinarum* honey and 334 mg/kg in *E. resinifera* honey (Table 2). Relative high amounts of Fe, Cu and Al were observed in *E. officinarum* honey (Table 2).

Table 2 Physicochemical parameters determined to honey samples

	Euphorbia resinifera	*Euphorbia officinarum*
pH	$4.10 \pm 0.0a$	$4.10 \pm 0.0a$
Moisture (%)	$18.6 \pm 0.0b$	$19.0 \pm 0.0a$
Free Acidity (meq/kg)	$10.1 \pm 0.8a$	$10.6 \pm 1.3a$
Lactonic Acidity (meq/kg)	$7.70 \pm 1.3b$	$11.0 \pm 0.9a$
Total Acidity (meq/kg)	$17.8 \pm 1.4a$	$21.6 \pm 2.2a$
Conductivity (μS/cm)	$379 \pm 0.6a$	$342 \pm 1.5b$
Diastase (Shade units/g)	$37.4 \pm 1.5a$	$13.2 \pm 1.3b$
Proline (mg/kg)	$954 \pm 36.5a$	$730 \pm 43b$
Ash (%)	$0.10 \pm 0.0a$	$0.10 \pm 0.0a$
HMF (mg/kg)	$2.30 \pm 0.3b$	$89.4 \pm 8.6a$
Colour Pfund scale (mm)	$408 \pm 1.9a$	$295 \pm 2.0b$
	Dark amber	**Dark amber**
Reducing sugars (%)	$66.6 \pm 2.3a$	$61.6 \pm 0.6\ b$
Fructose (g/100 g)	37.0 ± 0.8^a	34.9 ± 0.1^b
Glucose (g/100 g)	34.1 ± 3.8^a	30.2 ± 3.0^a
Sucrose (g/100 g)	5.50 ± 2.5^a	4.30 ± 0.2^a
Turanose (g/100 g)	2.10 ± 0.9^a	2.80 ± 0.4^a
Maltose (g/100 g)	2.30 ± 0.7^b	3.80 ± 0.2^a
Trehalose (g/100 g)	2.80 ± 0.5^a	4.00 ± 0.8^a
Mineral analysis (mg/kg)		
Fe	10.3 ± 0.3^b	333 ± 10.2^a
Zn	1.70 ± 0.2^a	1.80 ± 0.1^a
Mn	1.10 ± 0.0^b	1.50 ± 0.2^a
Cu	< LOD	110 ± 21.7^a
Al	11.9 ± 0.5^b	64.3 ± 6.8^a
Ca	117 ± 1.1^a	70.1 ± 0.4^b
K	334 ± 9.4^b	394 ± 0.1^a
Mg	40.2 ± 0.7^a	35.0 ± 2.2^b
Na	40.2 ± 0.1^a	36.9 ± 0.0^b
Polyphenols (mg GAE/100 g)	$54.5 \pm 1.7b$	$61.7 \pm 2.9a$
DPPH IC$_{50}$ (mg/mL)	$80.1 \pm 1.1a$	$55.5 \pm 0.7b$
Superoxide IC$_{50}$ (mg/mL)	$3.70 \pm 0.0a$	$2.80 \pm 0.2b$
Nitric oxide IC$_{50}$ (mg/mL)	$88.2 \pm 0.8b$	$116 \pm 1.4a$
Lipoxygenase IC$_{50}$ (mg/mL)	$32.7 \pm 0.4b$	$46.8 \pm 0.4a$
ACTI IC$_{50}$ (mg/mL)	$44.7 \pm 8.3b$	$165 \pm 8.5a$
Tyrosinase IC$_{50}$ (mg/mL)	$11.5 \pm 1.8b$	$54.9 \pm 3.2a$
Xanthine oxidase IC$_{50}$ (mg/mL)	$71.7 \pm 1.9b$	$94.9 \pm 0.4a$

Values in the same row followed by the same letter are not significantly different ($p < 0.05$) by Student's T test

[a] Represent the higher value

[b] Represent the low value

LOD (limit of detection) = 0.786 mg/ mL

Phenol content and antioxidant activity

The phenolic content in the *Euphorbia* honeys were as follows: *E. officinarum* honey (61.8 mg GAE/100 g) and *E. resinifera* honey (57.6 mg GAE/100 g) (Table 2).

Since honey contains several phenolic compounds with antioxidant capacity, it is interesting to evaluate the use of different antioxidant methods to test their antioxidant capabilities. Thus, three antioxidant tests were performed in this study (DPPH, superoxide and NO radicals scavenging capacity). In

the present work, *E. officinarum* honey presented lower IC_{50} values in the DPPH and superoxide radical scavenging activity (55.5 and 2.80 mg/mL, respectively) compared with *E. resinifera* honey (80.1 and 3.70 mg/mL, respectively) (Table 2), therefore with higher ability for scavenging those free radicals. In contrast, in what concerns the ability for scavenging the NO free radicals, *E. resinifera* honey presented lower IC_{50} value (88.2 mg/mL) than *E. officinarum* honey (116 mg/mL) (Table 2).

Inhibition of lipoxygenase, acetylcholinesterase, tyrosinase and xanthine oxidase activities

The inhibitory action of *E. resinifera* honey on lipoxygenase, acetylcholinesterase, tyrosinase and xanthine oxidase activities was better, with lower IC_{50} values (32.7, 44.7, 11.5 and 71.7 mg/mL, respectively), than *E. officinarum* honey, which exhibited higher IC_{50} values (46.8, 165, 54.9 and 94.9 mg/mL, respectively) (Table 2). These results showed a negative correlation between total phenols and IC_{50} values, that is, higher amounts of phenols promoted the enzyme inhibition.

Phenols' content and antioxidant activity of the aqueous extracts of *Euphorbia* plants

Generally, the aqueous extracts of *E. officinarum* had higher concentrations of total phenols than the ones of *E. resinifera* (Fig. 2), independently of the extraction time and the ratio plant material (d.w.)/volume of extraction solvent. The ratio mass of *E. officinarum*:solvent volume of 1:100 and extraction time of 1 h or 2 h provided extracts with higher concentrations of total phenols (11.8 mg GAE/g in both cases). In what concerns *E. resinifera*, the best extraction conditions were 1:100 and 30 min and 1 h (7.50 and 7.20 mg/g, respectively) (Fig. 2). Generally, and in both plant extracts, the lowest ratio originated extracts with lower amounts of phenols.

Antioxidant activity assayed through DPPH method, revealed that with the exception of the ratio 1:100, in the time periods of 30 min and 2 h, *E. resinifera* extracts had lower IC_{50} values (IC_{50} = 0.370 mg/mL), therefore higher capacity for scavenging the DPPH free radicals (Fig. 2b). Nevertheless, in what concerns the capacity for scavenging superoxide anion radicals, the best activity was found in *E. officinarum* extract of 1:100 ratio and after 1 h of extraction (IC_{50} = 0.17 mg/mL). In addition, 1 h of extraction originated extracts of *E. officinarum* with good ability for scavenging the superoxide radical anions, in opposition to the extracts of *E. resinifera* that showed the poorest superoxide scavenging activity (Fig. 2c). The best NO scavenging activity was found for *E. resinifera* extract of 1:100 ratio and after 2 h of extraction. Moreover, this time period provided the extracts of *E. resinifera* with the lowest IC_{50}, therefore with the best activities. Among the *E. officinarum* extracts, the ones yielded

using the ratio 1:100 for 1 h of extraction were the best (IC_{50} = 0.39 mg/mL) for scavenging the NO free radicals (Fig. 2d).

Enzymatic inhibitory activities of the aqueous extracts of *Euphorbia* plants

The extracts of *E. officinarum* showed the best anti-lipoxygenase activity (Fig. 3a) when the ratio was 1:100 and the extraction time was 2 h (IC_{50} = 0.57 mg/mL), immediately followed by those extracts with the ratio 1:100 and 1 h of extraction and 1:50 and 2 h of extraction (IC_{50} = 0.99 mg/mL and IC_{50} = 1.07 mg/mL, respectively). Nevertheless, this species also presented the worst activities in the following extracts (1:20 and 30 min of extraction; 1 h and 2 h of extraction) (Fig. 3a). In contrast to the inhibitory activity of lipoxygenase, in the inhibition action on acetylcholinesterase, the *E. resinifera* possess almost always better activities than *E. officinarum*, independently on the ratio or extraction time (Fig. 3b). The sole exception was the extract of 1:20 ratio and 30 min of extraction. The worst activities were found when the ratio was 1:20, independently on the species or time extraction (Fig. 3b). Generally, *E. resinifera* extracts presented better capacity for inhibiting tyrosinase activity, with only one exception (1:100 ratio and 1 h of extraction). In this case, *E. officinarum* had the lowest IC_{50} value (1.46 mg/mL), and therefore the best activity. This IC_{50} was similar to that observed for *E. resinifera* ratio 1:100 and 30 min of extraction (IC_{50} = 1.49 mg/mL). Generally, the trend of inhibitory activity was similar to that registered for acetylcholinesterase inhibitory activity, that is, the highest the ratio (plant material:solvent extraction), the best inhibitory activities were found (Fig. 3b and 3c). Concerning xanthine oxidase inhibitory activity, *E. officinarum* extracts obtained after 2 h of extraction and in all ratio assayed (1:20, 1:50 or 1:100) had the lowest IC_{50} values (23.6, 18.7 and 21.6 mg/mL, respectively), which means a best ability for inhibiting the xanthine oxidase activity (Fig. 3d). Regarding *E. resinifera*, only the extract 1:100 ratio and 2 h of extraction had significantly better activity than the remaining *E. resinifera* extracts (IC_{50} = 26.1 mg/mL) (Fig. 3d).

Comparison of studied activities between *Euphorbia* honey samples and aqueous *Euphorbia* extracts

When comparing the activities found in honey samples and those in aqueous extracts of the plants, the results observed for aqueous plant extracts were always better than those found in aqueous honey samples (Fig. 4a), which can partially be explained by the lowest amounts of phenols detected in honey samples (around 10–20 times lower than the plant extracts). However, in some cases, the activities of honey samples were not 10 or 20 times lower than the activities of plant extracts. For example, the minimal and maximal IC_{50} values of DPPH
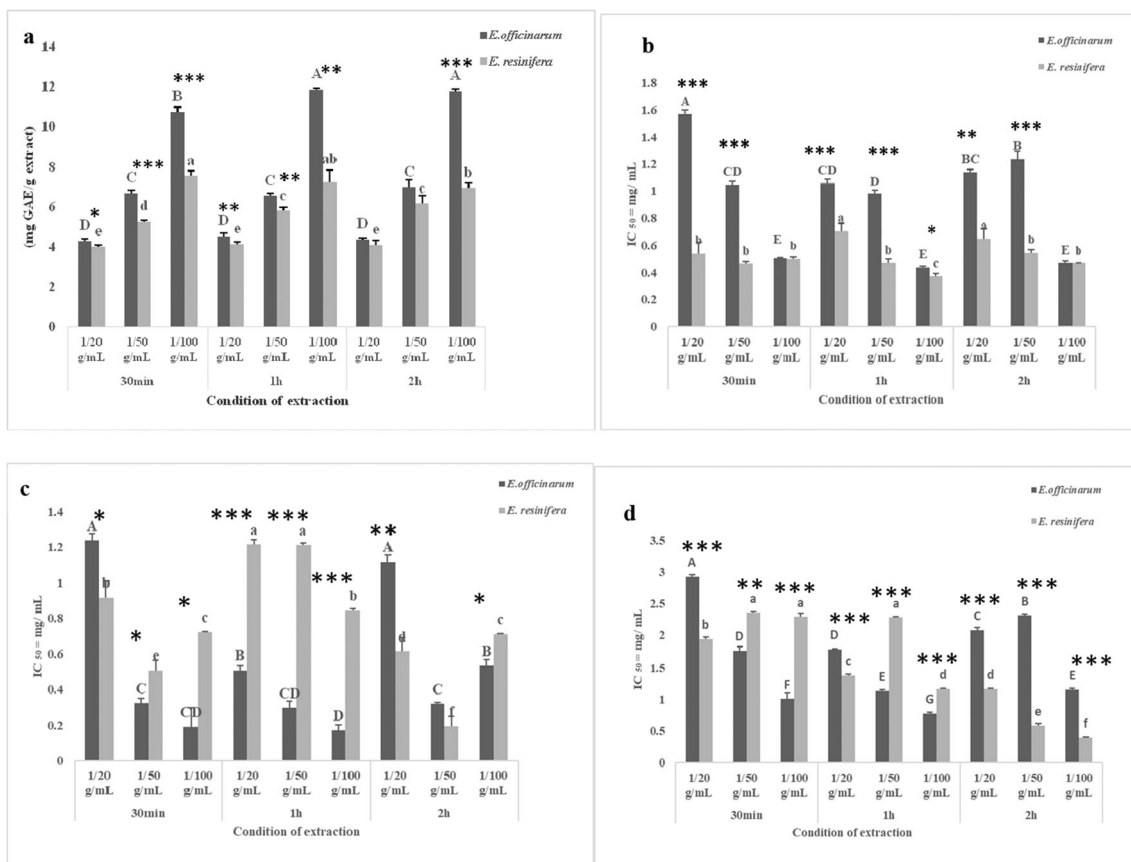
Fig. 2 Total phenols and antioxidant activities of *Euphorbia resinifera* O. Berg and *Euphorbia officinarum* L. water extracts. **a** Phenol compounds estimations. **b** DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity. **c** Scavenging ability of superoxide anion radical. **d** Nitric oxide (NO) scavenging activity. Capital letters present the statistical analysis of *E. officinarum* (p value < 0.005). Minimal letters present the statistical analysis of *E. resinifera* (p value < 0.005). p value by Student's T test: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$

and NO scavenging activities, and acetylcholinesterase inhibitory activity of *Euphorbia* plants extracts were 0.37 - 1.57; 0.39 - 2.92; and 0.70 - 8.36 mg/mL, respectively, which are more than 20 times lower than the respective IC_{50} values of honey (Table 2). These results mean that the activities of the plant extracts are more than 20 times greater than the activities of the honeys. Therefore, it might exist in some compounds, not determined in this work, present in honey samples that impair the biological properties. On the other hand, the capacity for scavenging the superoxide radical anions or the anti-acetylcholinesterase and anti-lipoxygenase activities of the aqueous *Euphorbia* extracts were approximately 10 times higher than those verified in the honey samples. On the anti-xanthine oxidase activity, the differences observed in plant extracts and honey samples were not as great as observed in the remaining biological properties (Fig. 4a).

When comparing *E. officinarum* and *E. resinifera* honey samples with those of the respective plant extracts, it was possible to verify that the activities of plant extracts were higher (blue colour means lower IC_{50}, thus higher inhibitory capacity or scavenging activity depending on the case) than honey samples. Xanthine oxidase inhibition activity of all

samples except 7, 8, 9 and 16 was poorer (higher IC_{50}) than the remaining activities (Fig. 4a). This permitted grouping these four samples in one cluster (Fig. 5a): the 2 samples of honey which stood out of the remaining samples by their weaker activities in almost all samples constituted another cluster; samples 3 and 15 constituted a third cluster with lower anti-xanthine oxidase activity than the remaining plant extracts but even superior to the honey extracts; and the remaining samples constitute a fourth cluster (Fig. 5a). According to the results, it seems that the extraction observed for *E. officinarum* (7, 8 and 9) and the extraction 16 for *E. resinifera* were the most adequate for obtaining better anti-xanthine oxidase.

Since the values of honey samples were very different from those obtained for plant extracts, only the plant extract parameters were considered in Fig. 4b. The matrix plot allowed concluding that *E. resinifera* extracts had better anti-acetylcholinesterase activity and DPPH free radical scavenging activity (Fig. 4b). The samples 7, 8, 9 and 15 (former 16) corresponded to the samples with the best anti-xanthine oxidase activity; samples 8 and 9 had also the best anti-lipoxygenase activity, along with sample 6. Samples 3, 6
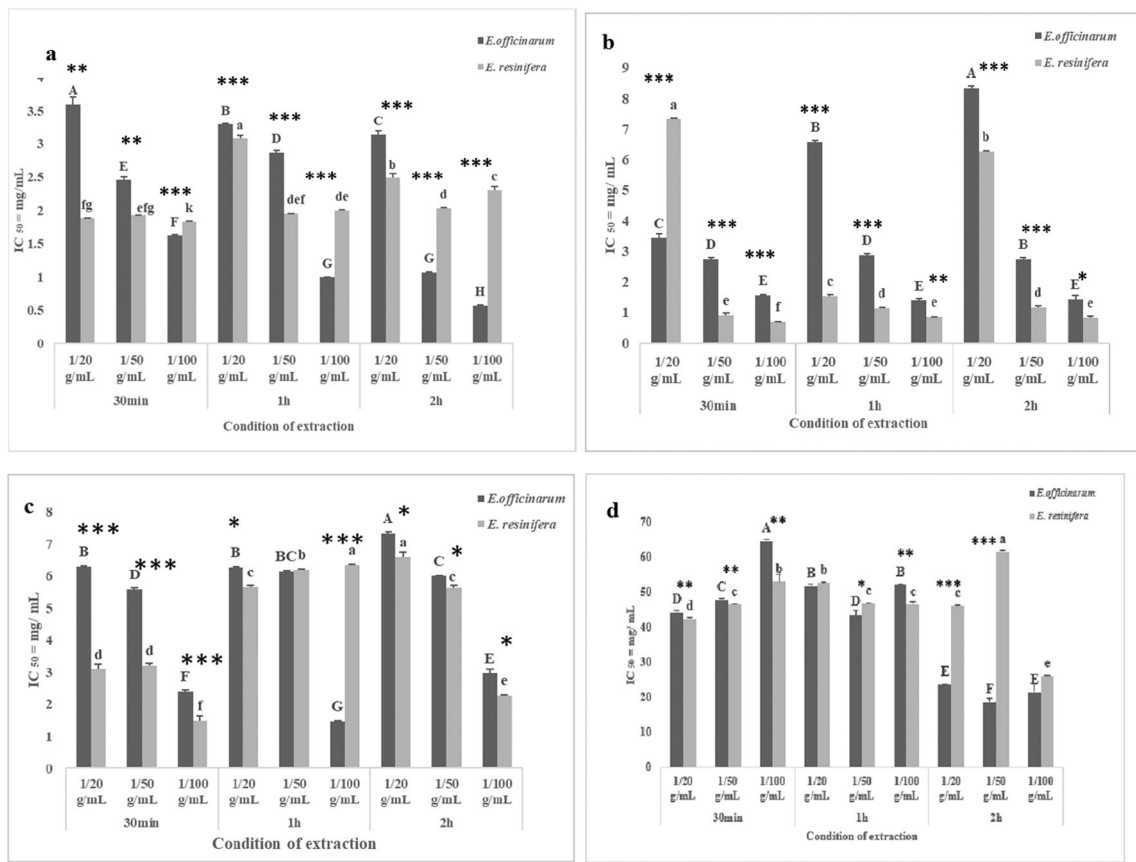
Fig. 3 Enzyme's inhibitory activities of *Euphorbia resinifera* O. Berg and *Euphorbia officinarum* L. water extracts. **a** Inhibition of lipoxygenase activities. **b** Inhibition of acetylcholinesterase activities. **c** Inhibition of tyrosinase activities. **d** Inhibition of xanthine oxidase activities. Capital letters present the statistical analysis of *E. officinarum* (p value < 0.005). Minimal letters present the statistical analysis of *E. resinifera* (p value < 0.005). p value by Student's T test: *p < 0.05, **p < 0.01, ***p < 0.001

and 9 (*E. officinarum*) and 15 and 18 had the best anti-tyrosinase activity (Fig. 4b). Figure 5b illustrates the dendrogram obtained only considering the values of plant extracts. In this case, the samples 7, 8, 9 and 15 (former 16) constitute a cluster as observed when honey samples were also

considered. It is the same for the samples 3 and 14 (former 15) that constitute other cluster (Fig. 5b).

Figure 6a depicts a plot of two principal components (PC1 and PC2) from all analyzed parameters for *Euphorbia* plant extracts and *Euphorbia* honeys. The
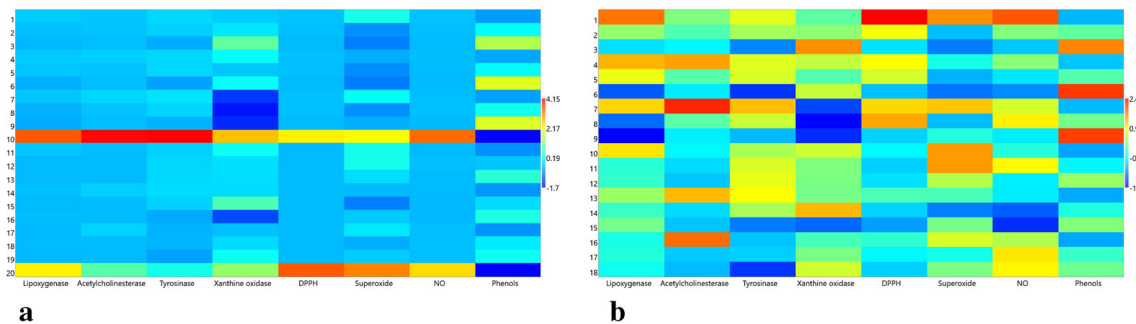
Fig. 4 **a** Two-dimensional plot of the data matrix (blue for the lowest value, red for the highest value) for inhibitory capacities of lipoxygenase, acetylcholinesterase, tyrosinase and xanthine oxidase (IC$_{50}$ values); for DPPH free radical, superoxide anion radical and nitric oxide (NO) scavenging abilities (IC$_{50}$ values) and total phenols (mg GAE/g). Legend: 1–9 - extracts of *E. officinarum* plants (1–3: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 4–6: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 7–9: 1/20, 1/50, 1/100 g/mL,

respectively, for an extraction period of 2 h); 11–19 - extracts of *E. resinifera* plants (11–13: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 14–16: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 17–19: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 10 and 20 - *E. officinarum* and *E. resinifera* honey samples, respectively. **b** Two-dimensional plot of the same data matrix as in Fig. 4a but without the honey samples (10 and 20)

a

b
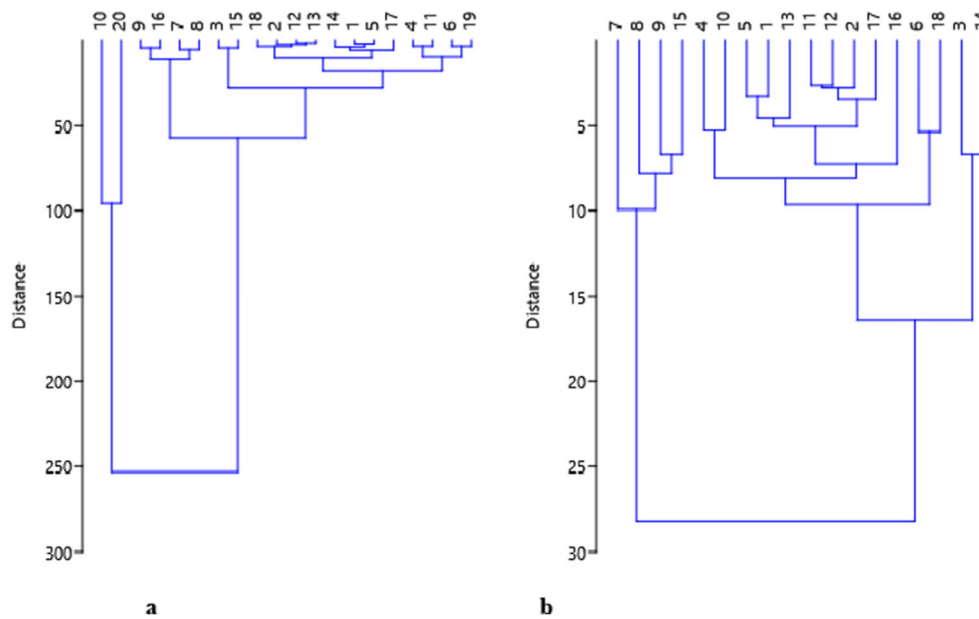
Fig. 5 a, b Dendrograms obtained by cluster analysis of the phenol content and activities, based on correlation and using Ward's method. **a** Legend: 1–9 - extracts of *E. officinarum* plants (1–3: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 4–6: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 7–9: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 11-19 - extracts of *E. resinifera* plants (11–13: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 14–16: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 17–19: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 10 and 20 - *E. officinarum*

and *E. resinifera* honey samples, respectively. **b** Legend: 1–9 - extracts of *E. officinarum* plants (1–3: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 4–6: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 7–9: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 10–18 - extracts of *E. resinifera* plants (10–12: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 13–15: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 16–18: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h)

figure shows 2 outliers corresponding to honey samples (10 and 20) occupying the positive part of the PC1. All plant extracts occupied the negative part of PC1. For this reason, those two honey samples were removed, and the data again analyzed (Fig. 6b). The two components represented 47.63% and 16.06% for PC1 and PC2, respectively. The plot shows that NO and DPPH scavenging activities are strongly related, as well as superoxide scavenging activity and anti-tyrosinase activity. The phenol content is strongly and negatively correlated with anti-lipoxygenase activity and less with anti-tyrosinase and anti-acetylcholinesterase activities, and superoxide, NO and DPPH scavenging activities. The samples 3, 6, 9; and 12, 15 and 18 occupied the negative part of PC1, that is, these samples of *E. officinarum* (3, 6, 9) and *E. resinifera* (12, 15, 18) all of them extracted using a ratio mass/volume (1/100), independently on the extraction time (1/2 h, 1 h and 2 h). In this negative part of PC1, were also placed the samples of *E. resinifera* (14 and 17) corresponding to the ratio 1/50 extracted for 1 h. In general, this PCA permitted distinguishing the samples with higher extraction efficiencies, with a ratio of 1/100, from the remaining extraction conditions, for both plant species (Fig. 6b).
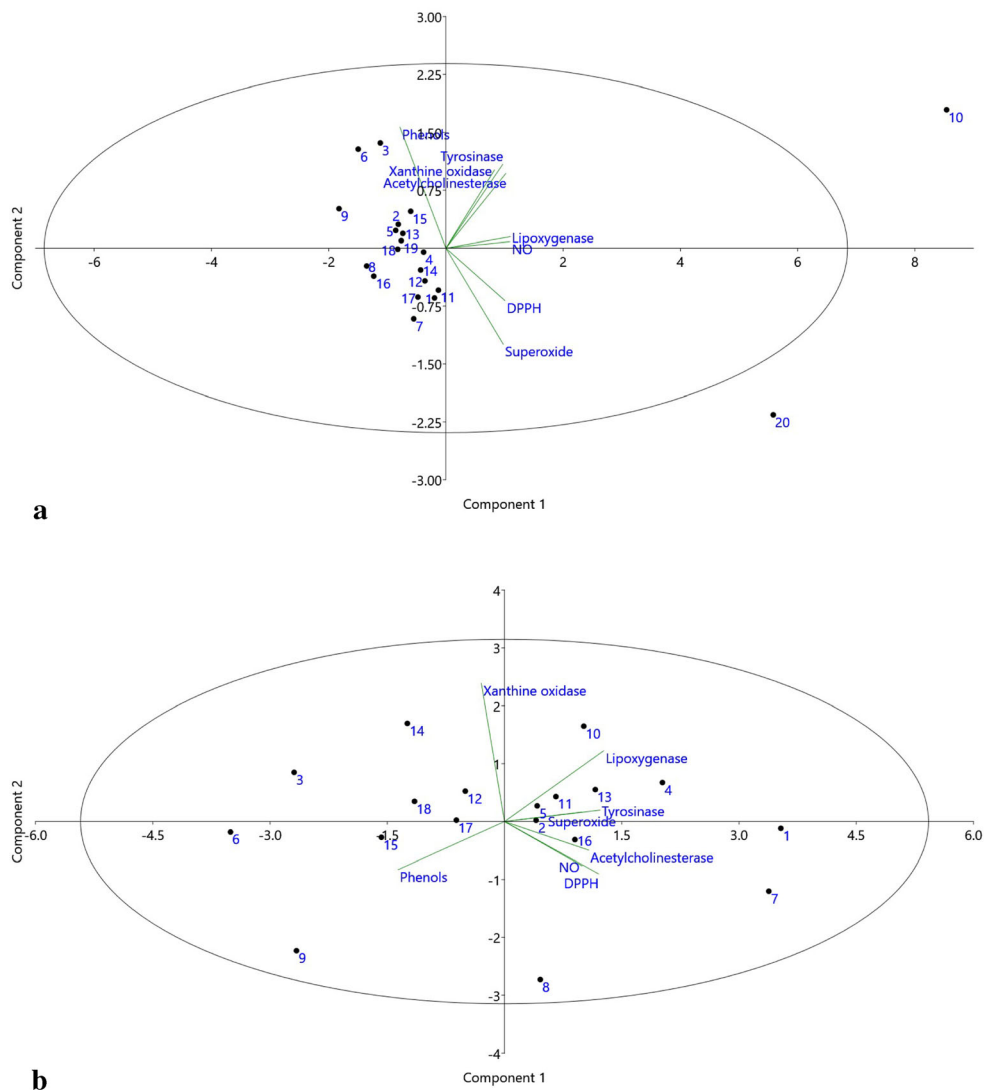
Discussion

Honey samples

General physicochemical properties

Physicochemical properties of honeys are important indicators of the quality and origin of this natural product (Tatjana et al. 2018). The pH is an important parameter during honeys' extraction and the storage, influencing the texture as well as the stability of the product (Bettara et al. 2015). Usually the pH of honey is < 4.0, which is an important factor preventing microorganisms' growth (Tatjana et al. 2018). In this study, pH values were similar to that found by Bettara et al. (2015) in eleven *E. officinarum* honey samples where pH values ranged from 4.0 to 3.8.

Organic acids are responsible for the acidity of honey and influence its taste (Tatjana et al. 2018). According to the values given by the Codex Alimentarius, the free acidity of honey must not exceed 50 milliequivalents of acid per 1000*g* (Codex Alimentarius Commission 1999). The free acidity values found in the present work were slightly lower than that previously reported (18.2 meq/kg for *E. resinifera* honey) (Chakir et al. 2016). In

Fig. 6 **a** Plot of two principal compounds analyses (PCA). Legend: 1–9 - extracts of *E. officinarum* plants (1–3: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 4–6: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 7–9: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 11–19 - extracts of *E. resinifera* plants (11–13: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 14–16: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 17–19: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 10 and 20 - *E. officinarum* and

E. resinifera honey samples, respectively. **b** Plot of two principal compounds analyses (PCA). Legend: 1–9 - extracts of *E. officinarum* plants (1–3: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 4–6: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 7–9: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h); 10–18 - extracts of *E. resinifera* plants (10–12: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 30 min; 13–15: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 1 h; 16–18: 1/20, 1/50, 1/100 g/mL, respectively, for an extraction period of 2 h)

another study (Naman et al. 2005), the results were significantly higher (33 meq/kg) than our results. Concerning *E. officinarum* honey, the results obtained by Bettara et al. (2015) were higher (53.22 meq/kg and 80.28 meq/kg) than our results for the same type of honey. In the work of Moujanni et al. (2018), higher lactonic acidity values ranged from 14.70 to 53.92 meq/kg (mean = 24.44 meq/kg) were reported for *E. resinifera* honey. On the other hand, the results found by Bettara et al. (2015) showed that *E. officinarum* honey presented lower lactonic acidity

value than the one we found in the present study (2.58 meq/kg).

Water content is the second most important parameter of honey and its content can vary from 15 to 23% (EU 2002). In our study, the values of moisture were within the standards proposed by the European legislation) (EU 2002). A previously published study (Naman et al., 2005) has shown that water content of *E. resinifera* was 20.00 and 18.00%. On the other hand, Bettara et al. (2015) has found 18.50% for *E. officinarum*, a value similar to that one found in the present work (19%).

The electrical conductivity of honey usually depends on the content of minerals, organic acids and protein (Tatjana et al. 2018). The results of electrical conductivity found in this work are in agreement with the values proposed by the European legislation (< 800 μS/cm) (EU 2002). In another study (Chakir et al. 2016), *E. resinifera* honey showed values (761 μS/cm) higher than the ones of this work. For *E. officinarum* honey, a study performed by Bettara et al. (2015) has also shown a value superior (561.18 μS/cm) than the one we found.

The predominant enzyme in honey is diastase which allows the degradation of starch to maltose; this enzyme is relatively sensitive to heat and storage (Tatjana et al. 2018). The diastase activity is one of the important indicators of storage condition; it is used to confirm the honey freshness (Chakir et al. 2016). Both honey samples were above the minimum required by European legislation (2002), which is in agreement with the values reported by Chakir et al. (2016) for honeys produced from different plants in Morocco.

The amino acids' content assessed as proline content of honey varied between 0.05 and 0.1%, being proline the most abundant (Tatjana et al. 2018). Both samples contained more than the minimum acceptable for proline concentration (200 mg/kg) (Hermosin et al. (2003).

The ash content, as reported by Moujanni et al. (2018), is traditionally used to determine the honey type (nectar or honeydew). According to the European legislation (2002), the value of ash content in honey must not exceed 0.6%, whereby the honey samples with values lower than this percentage is not a honeydew.

The HMF (hydroxymethylfurfural) is a compound formed slowly during honey natural storage and quickly when the honey is heated (Tatjana et al. 2018). The Codex Alimentarius (1999) requires that the HMF content must not exceed 40 mg/kg in honey. Concerning *E. officinarum* honey, the value surpasses the limit permitted by the European regulations. According to Bettara et al. (2015), *E. officinarum* honey had 85.48 mg/kg, that is, close to the value that we found. Regarding *E. resinifera* honey, the value found was 2.30 mg/kg (< 40 mg/kg), a value that meets the prescribed standards of the Codex Alimentarius (1999). In a study presented by Moujanni et al. (2018), *E. resinifera* honeys showed lower values of HMF ranging from 0.4 to 16.8 mg/kg.

Honey colour is an indirect indicator of its content of polyphenols, terpenes and carotenoids (Elamine et al. 2017). Both samples had a mm Pfund > 114, corresponding to a dark amber colour.

The reducing sugar content in honey varies due to storage conditions, enzymatic activity, the reversal of acids and harvest period (Aljohar et al. 2018). The European standard for a quality honey recommends values above 60% for content of reducing sugars (fructose, glucose and maltose) (EU 2002); therefore, both samples are within the range of the quality

standards. Honey consists mostly of the monosaccharides glucose and fructose (Mondragon-Cortez et al. 2013). According to Chis et al. (2012), the fructose/glucose ratio is able to influence the crystallization of honey, and more precisely crystallization is prevented by fructose, but promoted by glucose. This ratio was 1.08 for *E. resinifera* honey and 1.15 for *E. officinarum* honey (Table 2). Such results may indicate that *E. resinifera* honey is more likely to crystallize than *E. officinarum* honey. The amounts of these two monosaccharides were within the range found by Moujanni et al. (2018) for *E. resinifera* honey.

According to Elamine et al. (2019), potassium prevails in honey samples followed by calcium and sodium. In the present work, this was observed only for *E. resinifera* honey (Table 2), while for *E. officinarum* honey, the second and third elements were Fe (332.5 mg/kg) and copper (109.7 mg/kg). The high amounts of Fe, Cu and Al in *E. officinarum* honey may be related to some environmental contamination by these elements in the area surrounding beehives or even the inadequate use of metallic containers for honey storage, although the importance of the origin area on the elemental composition of honeys (Squadrone et al. 2020).

Phenol content and antioxidant activity

Generally, and following research done on the Sahara honeys, the total phenolic contents found ranged between 72.0 and 97.9 mg GAE/100 g of honey (Moussa et al. 2015). In another study done on *Euphorbia* Turkish honey (Gül and Pehlivan 2018), the authors reported 278.98 mg GAE/100 g, a value higher than those found in the present work. In another work (Aazza et al. 2014), where the authors studied several types of Moroccan honeys, much higher phenolic content (518.92 mg GAE/100 g) was found for *Euphorbia resinifera* honey when compared with the results of this work (Table 2).

Concerning the capacity for scavenging free radicals, our data are in close agreement with Kıvrak and Kıvrak (2016) who have found DPPH IC_{50} in the range of 24.46–81.82 mg/mL for Turkish *Euphorbia* honeys. Nevertheless, some authors (Bouhlali et al. 2016) showed better capacity for scavenging DPPH free radical of *E. resinifera* honey (IC_{50} = 7.91 mg/mL) than our sample (Table 2). The results reported by Elamine et al. (2017) have shown that the capacity for scavenging the superoxide anion radicals of Moroccan *Bupleurum spinosum* honey, with the highest IC_{50} value (51.31 mg/mL), was weaker than that of Moroccan *Euphorbia* honey. The IC_{50} values found for the NO assay were similar to those already reported (Aazza et al. 2014) for *E. resinifera* honey (IC_{50} = 95.14 mg/mL) and for Moroccan *B. spinosum* honey (IC_{50} = 118.38 mg/mL) (Elamine et al. 2017). These differences observed can be attributed to the botanic origin as well as the climatic conditions where samples were collected (Elamine et al. 2017; De Sousa et al. 2016). Higher amounts

of phenols in *E. officinarum* honey determined its better ability for scavenging DPPH and superoxide radical anions (lower IC_{50} values), nevertheless with a negative effect on the ability for scavenging NO free radicals, as revealed by the best activity found in *E. resinifera* honey (Table 2).

Inhibition of lipoxygenase, acetylcholinesterase, tyrosinase and xanthine oxidase activities

The negative correlation between total phenols and IC_{50} values, that is, higher amounts of phenols promoted the enzyme inhibition, is expected and even observed in some cases for natural products (El-Guendouz et al. 2016). The biological activities cannot solely be attributed to the phenols, even to individual ones, when we are working with complex systems such as food matrices in which honey is included. Several components other than phenols in different proportions can contribute to the properties found. Moreover, such components and their relative proportions can change depending on uncontrolled several factors (e.g. climate, soil…). In addition, only two samples of honey were studied; therefore and although one of them presented better results than the other one, it is just better to say that both present capacity for inhibiting those enzymes. Much more samples would be needed to be sure about the differences observed in the present work. However, it will be always difficult due to the limited areas of *E. resinifera* honey production.

Other types of monofloral honeys (citrus, lavender) or different geographical origins have been reported as possessing anti-lipoxygenase activity (Silva et al. 2016), anti-acetylcholinesterase activity (Philip and Fadzelly 2015), anti-tyrosinase activity (Di Petrillo et al. 2018; Jantakee and Tragoolpua 2015) and anti-xanthine oxidase activity (Di Petrillo et al. 2018; Sahin 2015).

Phenols' content and antioxidant activity of the aqueous extracts of *Euphorbia* plants

Generally, and in both plant extracts, the lowest ratio originated extracts with lower amounts of phenols: the higher the ratio, the higher the phenol concentration. These results may reveal that lower volume of water (extraction solvent) was not enough for extracting higher amounts of phenols; probably the components extracted had low solubility in water (e.g. aglycones) which were only extracted and, therefore, solubilized in higher volumes of water. For this reason, other authors (Farah et al. 2014) had found better extraction results than those found in the present work, but using other extraction solvent (ethyl acetate).

The lowest values of IC_{50} found in the present work for the three assays (DPPH, superoxide and NO scavenging activity)

were always higher than those verified for the standards used for comparison ($IC_{50} = 0.022$, $IC_{50} = 0.013$ and $IC_{50} = 0.229$ mg/mL, for BHT, ascorbic acid and curcumin, respectively). With the exception of the IC_{50} value for curcumin, which was approximately the half of that verified for the best extract ($IC_{50} = 0.393$ mg/mL), the remaining standards were approximately ten times lower than the best extracts for scavenging DPPH or superoxide free radicals. The capacity for scavenging DPPH free radicals reported by Basma et al. (2011) for methanolic extracts of *E. hirta* leaves (1 mg/mL had a percentage of inhibition of 73%) was poorer than that observed in the present work. However, for methanolic extract of *E. resinifera* roots, Farah et al. (2014) described significantly higher activity ($IC_{50} = 0.010$ mg/mL).

Enzymatic inhibitory activities of the aqueous extracts of *Euphorbia* plants

The capacity for inhibiting the xanthine oxidase activity in the present work was much lower than those verified for extracts obtained using other solvents and from other species of *Euphorbia* ($IC_{50} = 0.1$ mg/mL) (Chen et al. 2009; Nguyen et al. 2004).

Galantamine, kojic acid and nordihydroguaiaretic acid (NDGA) were used as standards to compare their activities with the ones of the samples, and all of them presented significantly better activities than the sample extracts, presenting lower IC_{50} values ($IC_{50} = 0.001$, 0.006 and 0.003 mg/mL, respectively).

The importance of phenols for the antioxidant activity of the extracts, particularly for the capacity for scavenging DPPH free radicals, is evident in both species extracts, through the negative correlation between the content phenols and the IC_{50} values (Table 3), that is, the highest concentrations of phenols the best scavenging DPPH free radicals. The same can be observed in what concerns the inhibition of lipoxygenase and acetylcholinesterase activities; nevertheless, the role of phenols on the inhibitory action on xanthine oxidase is absent in both extracts (Table 3). Non-phenolic compounds can be responsible for the xanthine oxidase inhibitory activities of the extracts. The correlation between the phenol content and the capacity for scavenging NO and superoxide radicals or between the phenol content and the inhibitory activity on tyrosinase was also negative in both extracts, nevertheless only with statistical significance in the *E. officinarum* extracts. Therefore, particularly in the case of *E. resinifera* extracts, not just phenols but also other components play an important role on the properties detected. When both data of *E. officinarum* and *E. resinifera* are treated as a whole, only the correlation between phenol content and anti-xanthine oxidase activity is

Table 3 Pearson correlation coefficients between total phenols and antioxidant activities and enzyme inhibitory activities of *Euphorbia* extracts

	Phenol	Lipoxygenase	Acetylcholinesterase	Tyrosinase	Xanthine oxidase	DPPH	NO	Superoxyde
E. officinarum	1	− 0.878**	− 0.774**	− 0.949**	0.145	− 0.901**	− 0.778**	− 0.622**
E. resinifera	1	− 0.464*	− 0.697**	− 0.356	− 0.235	− 0.704**	− 0.163	− 0.308
E. officinarum *E. resinifera*	1	− 0.756**	− 0.531**	− 0.629**	− 0.030	− 0.413**	− 0.578**	− 0.452**

**Correlation is significant at the $p < 0.01$ level

*Correlation is significant at the $p < 0.05$ level

nonexistent, as expected since when the results were treated separately, they did not show any correlation type (Table 3).

Conclusion

The results of this work have shown that *Euphorbia officinarum* and *Euphorbia resinifera* honeys were within the limits established by the European legislation. However, *Euphorbia officinarum* honey revealed high HMF content, which may indicate inadequate heating and/or storage conditions. The amounts of iron, copper and aluminium were also detected in relatively high amounts in *E. officinarum* honey, which may be related to the environmental pollution around the beehives or inadequate storage. Such results suggest the need to improve the production and storage conditions of honey. In addition, and in what concerns the extracts of plants visited by bees (*E. resinifera* and *E. officinarum*), it highlights the importance of refining extraction procedure for *Euphorbia* plant: the volume of solvent, the ratio and the extraction time in order to obtain aqueous extracts with better in vitro antioxidant and enzyme inhibitory properties (anti-lipoxygenase, anti-acetylcholinesterase and anti-tyrosinase). The ratio plant mass:solvent volume (1:100) and extraction time (1 - 2 h) were associated with higher total phenols and better antioxidant activities and lipoxygenase, acetylcholinesterase and tyrosinase inhibitory activities, regardless of the plant species. Lastly, the study suggests that the differences in the activities found between the honey samples (lower activity) and *Euphorbia* extracts cannot be attributed just to the levels of phenols in the samples since the difference in concentrations of these compounds in both samples was not proportional to the differences found in the in vitro activities.

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