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Bioactive compounds and antibacterial properties of monofloral Ulmo honey

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ABSTRACT

Monofloral Ulmo honey is a very appreciated product in the international market but more information is needed in order to support their health properties. Total phenolic and flavonoid contents, antioxidant capacity and antibacterial activity of monofloral Ulmo (*Eucryphia cordifolia*) honey samples were determined. The samples contained between 176 and 208 mg gallic acid eq (GAE)/100 g for total phenolic content (TPC) and 43–90 mg Quercetin eq/100 g for total flavonoid content (TFC). The antioxidant activity ranged between 91 and 152 mM eq Trolox/g and 28–49 mM eq Trolox/g for DPPH and ABTS assays, respectively. All phenolic extracts from honey samples inhibited *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Escherichia coli* by plate assays. The chromatographic profile showed that Ulmo honey contained the polyphenolic gallic, caffeic, and coumaric acids, the flavonoids pinocembrin, chrysin, quercetin, luteolin and apigenin, and abscisic acid.

Compuestos bioactivos y propiedades antibacterianas de la miel monofloral de Ulmo

RESUMEN

La miel monofloral de Ulmo es un producto muy apreciado en el mercado internacional, pero se necesita más información para respaldar sus propiedades saludables. Se determinaron los contenidos fenólicos y flavonoides totales, la capacidad antioxidante y la actividad antibacteriana de muestras de miel monofloral de Ulmo (*Eucryphia cordifolia*). Las muestras contenían entre 176 – 208 mg eq de ácido gálico (GAE)/100 g para el contenido fenólico total (TPC) y 43 – 90 mg eq de quercetina/100 g para el contenido total de flavonoides (TFC). La capacidad antioxidante osciló entre 91 – 152 mM eq Trolox/g y 28 – 49 mM eq Trolox/g, para los ensayos de DPPH y ABTS, respectivamente. Todos los extractos fenólicos de muestras de miel de ulmo inhibieron *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* y *Escherichia coli* mediante ensayos en placa. El perfil cromatográfico mostró que la miel de ulmo contenía como compuestos bioactivos los ácidos, gálico, cafeico y cumárico, los flavonoides pinocembrina, crisina, quercetina, luteolina y apigenina, y ácido abscísico.

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1. Introduction

Honey is the natural product obtained after flora nectar is collected, transformed, and stored by honey bees (*Apis mellifera* L.). There are different types of honey according to the floral source from which the bees have collected nectar. Honey is usually classified according to the floral pollen present in the sample (Anklam, 1998).

There is a close relationship between the botanical origin of honey and its nutritional, antioxidant, and antimicrobial properties. For example, black forest honey has a lower antioxidant content than buckwheat honey (USDA, 2016), while monofloral Manuka honey from the tree *Leptospermum scoparium* is recognized worldwide for its nutraceutical properties (Alzahrani et al., 2012). Identification of the flora source of honey associates the presence of certain attributes to a particular honey.

In Chile, different types of honeys, with particular qualities and characteristics, have been discriminated based on the different climate zones, which affect the floral profile and floral abundance (Montenegro, Santander, Jara, Nuñez, & Fredes, 2013). One species with flowers that are very attractive to the

bee is Ulmo (*Eucryphia cordifolia* Cav.). Recently, Ulmo honey has gained attention for its antioxidant and antimicrobial properties. *In vivo* studies have shown that Ulmo honey is able to activate the monocytic response through regulation of anti-inflammatory factors (Jull, Rodgers, & Walker, 2008; Tonks et al., 2003) that allow the regeneration of epithelial cells in burn wounds (Schencke, Salvo, Vasconcellos, & Del Sol, 2013). Ulmo honey was reported to have a greater inhibitory activity against *E. coli*, *Pseudomonas aeruginosa* and five types of methicillin-resistant *Staphylococcus aureus* (MRSA) than Manuka honey (Sherlock et al., 2010). More recently, Acevedo et al. (2017) reported activity of Ulmo honey on these bacteria strains as well as *Aeromonas hydrophilia*, *Klebsiella pneumoniae* and *Salmonella enteric*. However, both studies used limited honey samples and did not report the botanical origin of the honey.

These regenerative and antimicrobial activities are mainly attributed to the phenolic compounds present in honey, such as flavonoids, benzoic acid derivatives and volatile compounds (Pita-Calvo & Vázquez, 2017). *P*-coumaric, ferulic, chlorogenic, and caffeic acids have been identified in Ulmo

honey, as well as the flavonoids kaempferol and luteolin (Velásquez et al., 2017) along with some volatile ketones (Montenegro et al., 2009). However, there is a knowledge gap, since no studies have shown a relationship between the botanical origin, the polyphenol profile, and the antioxidant and antimicrobial properties of Ulmo honey.

The current study aimed to analyze monofloral Ulmo honey samples, those with high pollen content from *Eucryphia cordifolia*, and to identify the potential molecules responsible for its reported medicinal properties.

2. Materials and methods

2.1. Samples

Samples were purchased from local beekeepers from the Mediterranean climate area of Southern Chile (X Región de Los Lagos). Only honeys with $\geq 45\%$ of *Eucryphia cordifolia* pollen determined by a melissopalynological analysis were selected.

2.2. Melissopalynological analysis

The botanical origin of the honeys was determined according to Chilean Standard Normative 2981 (Montenegro, Gómez, Díaz-Forestier, & Pizarro, 2008). Briefly, 10 g of honey were diluted in 10 mL of distilled water, centrifuged at 2500 rpm for 5 min, and the sediment was re-suspended in 0.1 mL of distilled water. Optical microscopy was used to observe the pollen grains and were identified using a palinoteque. Only monofloral honeys, with a composition equal or higher than 45% of a unique plant species, were considered.

2.3. Ulmo honey extracts

Samples were diluted in acidic water (pH 2 with HCl). The dissolution was put into an open chromatographic column (250 mm x 20 mm) filled with amberlite and washed with acidic water, distilled water and ethanol. Collected extracts were concentrated with a vacuum rotary evaporator until dryness (Buchi, Flawil, Switzerland), dissolved in distilled water, filtrated and stored at -20°C until use. Controls were carried out to ensure the innocuity of the extracts before doing the tests.

2.4. Total phenolic content (TPC)

The phenolic content of the samples was evaluated by Folin-Ciocalteu (FC) assay based on Bridi et al. (2014). Five hundred microliters of honey samples diluted in water (0.2 g honey to 4 mL of water) were mixed with 2.0 mL of the FC reagent 1:10 (v/v) and 1.2 mL of a solution of Na_2CO_3 . The absorbance at 760 nm was measured after 60 min at room temperature in the dark, using an Agilent 8453 UV-visible spectrophotometer (Palo Alto, Calif., USA). A calibration curve constructed with gallic acid was used to quantify and results are expressed as mg GAE/100 g honey.

2.5. Total flavonoid content (TFC)

Total flavonoids were determined using an aluminum chloride method based in Woisky and Salatino (1998) procedure. Five hundred microliters of honey sample diluted in water (0.1 g honey to 1.6 mL of water) were mixed with 500 μL of

2% AlCl_3 ethanol solution. After 60 min at room temperature, the absorbance at 420 nm was measured using an Agilent 8453 UV-visible spectrophotometer (Palo Alto, Calif., USA). Total flavonoids content was calculated as mg QE/100 g honey, from a calibration curve.

2.6. 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)

The scavenging activity was determined based on Thaipong, Boonprakob, Crosby, Cisneros-Zevallos, and Byrne (2006) procedure. Twenty-four milligrams of DPPH were dissolved in 100 mL of methanol. Ten milliliters of the solution were diluted with methanol until an absorbance of 1.1 ± 0.02 at 515 nm is obtained. Five hundred microliters of aqueous honey dilution reacted with 4,5 mL of final DPPH solution in the dark for 30 min, and the absorbance at 517 nm was measured. A calibration curve with TROLOX was employed and the results were expressed in mg eq TROLOX/g honey.

2.7. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging activity (ABTS⁺)

The scavenging activity of the samples measured on $\text{ABTS}^{\cdot+}$ radical was based on the method described by Van den Berg, Haenen, van den Berg, and Bast (1999), with some modifications. Twenty milligrams of potassium persulfate were added to 20 mL of 7 mM ABTS aqueous solution. The mixture was incubated at darkness between 12 and 16 h and then diluted with ethanol until final absorbance of 0.70 ± 0.02 at 732 nm. Fifty microliters of aqueous sample dilution were mixed with 1.45 mL of reagent solution and absorbance at 732 nm was measured after 30 min in the dark. Calibration curve was constructed with and the results were expressed in equivalent mg Trolox/g honey.

2.8. Phenolic profile by HPLC-MS-MS

An ABSciex triple Quad 4500 mass spectrometer equipped with an electrospray (TurboV) interface coupled to an Eksigent Ekspert Ultra LC100 with Ekspert Ultra LC100-XL autosampler system (AB/Sciex Concord, Ontario, Canada) was used in order to identify polyphenols in the samples. A LiChrospher 100 RP-18 endcapped column (125 mm x 4 mm id, 5 μm) (Merck, Darmstadt, Germany) and a mobile phase of 0.1% formic acid in water (A) and methanol (B) with the following gradient composition: 0–1 min, 15% B; 1–17 min, 15–100% B; 17–21 min 100–100% B; 21–22 min, 100–15% B; 22–25 min, 15–15% B, were employed. Ten microliters of sample at 0.5 mL/min with a controlled temperature of 30°C were applied. Quantification was performed with calibration curves using commercial available standards.

2.9. Antibacterial activity

Bacteria strains *E. coli* ATCC-25922, *S. aureus* ATCC-25923, *Pseudomonas aeruginosa* ATCC-15692, *Streptococcus pyogenes* were used. The strains were propagated on Mueller Hinton agar 24 h in advance. Colonies were selected and diluted in saline solution to ensure a concentration of viable 10^8 CFU/mL by visual comparison with a 0.5 McFarland standard (Becton Dickinson and Company, USA). With a swab, strains were placed on petri dishes with Mueller Hinton agar, and 6 mm

diameter holes were made and filled with 100 μ L of honey dilution. The Petri plates were incubated for 24 h at 35°C. Then, the inhibition diameter that appeared around each hole was measured (CLSI, 2006).

2.10. Statistical analysis

Statistical analyses of the results were performed using a variance analysis by Tukey's HSD method with 95% of confidence computed by STATGRAPHICS Centurion XV software 15.02.05. All tests were carried out in triplicate, and the results are reported as the means and standard deviation. Correlations between the analyses performed were made using the Pearson procedure ($p < 0.05$). In order to evaluate the general effect of the determined parameters, the principal component analysis, PCA (The Unscrambler 9.7 software, CAMO AS, Oslo, Norway) methods was chosen.

3. Results and discussion

3.1. Botanical origin

The antioxidant and antimicrobial activities of Chilean honey have been studied over the last few years. These properties have been mainly attributed to the phenolic compounds present in the samples (Bridi et al., 2015; Giordano et al., 2018; Montenegro et al., 2013). However, these honey samples not included the polyphenolic profile of several Ulmo honey samples and its relation with the antibacterial activity.

Eight monofloral Ulmo honey samples were collected in the X Los Lagos region of southern Chile. The botanical origin was determined by analysis of pollen grain present in the honeys, according to the official policy established by the Standards Division of the National Institute for Standardization (Montenegro et al., 2008), which classifies the honeys as monofloral, bifloral, or polyfloral (Montenegro et al., 2009). Monofloral honeys are those in which at least 45% or more of the pollen grains belong to the same species. The eight evaluated samples contained between 86.6% and 96.8% *Eucryphia cordifolia* pollen grain (Table 1). Pollen analysis showed a low diversity of secondary species, with contributions from Arrayán (*Luma apiculata*), Tineo (*Weinmannia trichosperma*) and alfalfa chilota (*Lotus pedunculatus*).

3.2. Phenolic and flavonoid contents and antioxidant properties

The total phenolic (TP) content of the honey samples ranged between 176 and 208 mg GAE/100 g honey (Figure 1(a)). Similar values among the samples were expected since all were Ulmo

monofloral honeys, as phenolic composition in honey depends strongly on the plant species from which the nectar was collected (Biesaga & Pyrzyńska, 2013). All Ulmo honey samples contained at least twice the amount of phenols reported for Manuka honey ($83,19 \pm 12,03$ mg GAE/100 g) and seven to eight times more than Sesame honey ($31,49 \pm 1,68$ mg GAE/100 g of honey) (Jantakee & Tragoolpua, 2015). Likewise, the TP contents of the Ulmo honey samples were higher than those reported for honeys sourced from rosemary, orange, thyme (Alves, Ramos, Gonçalves, Bernardo, & Mendes, 2013); mint (Boussaid et al., 2018); chestnut, blackberry, honeydew (Escuredo, Míguez, Fernández-González, & Seijo, 2013); and citrus (Perna, Simonetti, Intaglietta, Sofo, & Gambacorta, 2012). Even the TP content of Ulmo honey were higher than several TP contents of fruits and flowers (Mahmoudi et al., 2019; Wang, Clardy, Hui, Gao, & Wu, 2019).

Among the Ulmo honey samples, samples 4 and 5 had significantly higher TPC compared to samples 3 and 6. The TP content was higher when the secondary species of nectar origin was Tineo, and lower when it was alfalfa chilota or Arrayán.

The total flavonoid (TF) content of the Ulmo honey samples ranged from 40 to 90 mg QE/100 g of honey, with an average of $75,50 \pm 14,45$ mg QE/100 g (Figure 1(b)). The average TF content of Ulmo honey was about 10 times higher than that of Sesame honey ($5,50 \pm 0,02$ mg QE/100 g of honey), and four to five times higher than Manuka honey ($16,29 \pm 0,12$ mg quercetin/100 g of honey) (Jantakee & Tragoolpua, 2015). Sample 5 showed a statistically different TF content from the rest of the samples, presenting the lowest flavonoid content, however, it seems unrelated to the botanical origin.

The antioxidant activities of the Ulmo honey samples were evaluated using two different antioxidant assays, the ABTS and DPPH assays, with Trolox as the standard (Figure 1(c)). Honey samples 1, 2 and 8 showed a significantly higher antioxidant capacity when evaluated using the DPPH radical. The samples with the highest DPPH scavenging activity were also sourced from Arrayán, while the others did not. The presence of Arrayán nectar could indicate that these species increases the antioxidant activity of the Ulmo honeys. In the ABTS assay, samples 1 and 5 showed the highest antioxidant activities. Overall, the samples showed a three-fold higher value when evaluated using DPPH (up to 152 mM eq Trolox/g honey) compared to when using ABTS radicals (up to 49 mM eq Trolox/g honey). These results are contrary to those reported by Attanzio, Tesoriere, Allegra, and Livrea (2016), who found higher values using ABTS compared to DPPH when evaluating Sicilian monofloral honeys.

For a better characterization of the Ulmo honey samples (Pita-Calvo & Vázquez, 2017) liquid chromatography was used to analyze the polyphenol content. All samples exhibited

Table 1. Botanical origin and classification of Ulmo honey samples.

Tabla 1. Origen botánico y clasificación de las muestras de miel de Ulmo.

Sample	Ulmo (%)	Secondary species (%)	Secondary species (Scientific name)	Secondary species (Common name)	Code name
1	96.8	1.9	<i>Luma apiculata</i>	Arrayán	U-Ar1
2	95.8	1.6	<i>Luma apiculata</i>	Arrayán	U-Ar2
3	94.5	2.5	<i>Lotus pedunculatus</i>	Alfalfa chilota	U-Alf3
4	94.2	3.8	<i>Weinmannia trichosperma</i>	Tineo	U-T
5	91.6	2.9	<i>Lotus pedunculatus</i>	Alfalfa chilota	U-Alf5
6	91.0	2.6	<i>Lotus pedunculatus</i>	Alfalfa chilota	U-Alf6
7	86.7	7.7	<i>Lotus pedunculatus</i>	Alfalfa chilota	U-Alf7
8	86.6	6.2	<i>Luma apiculata</i>	Arrayán	U-Ar8

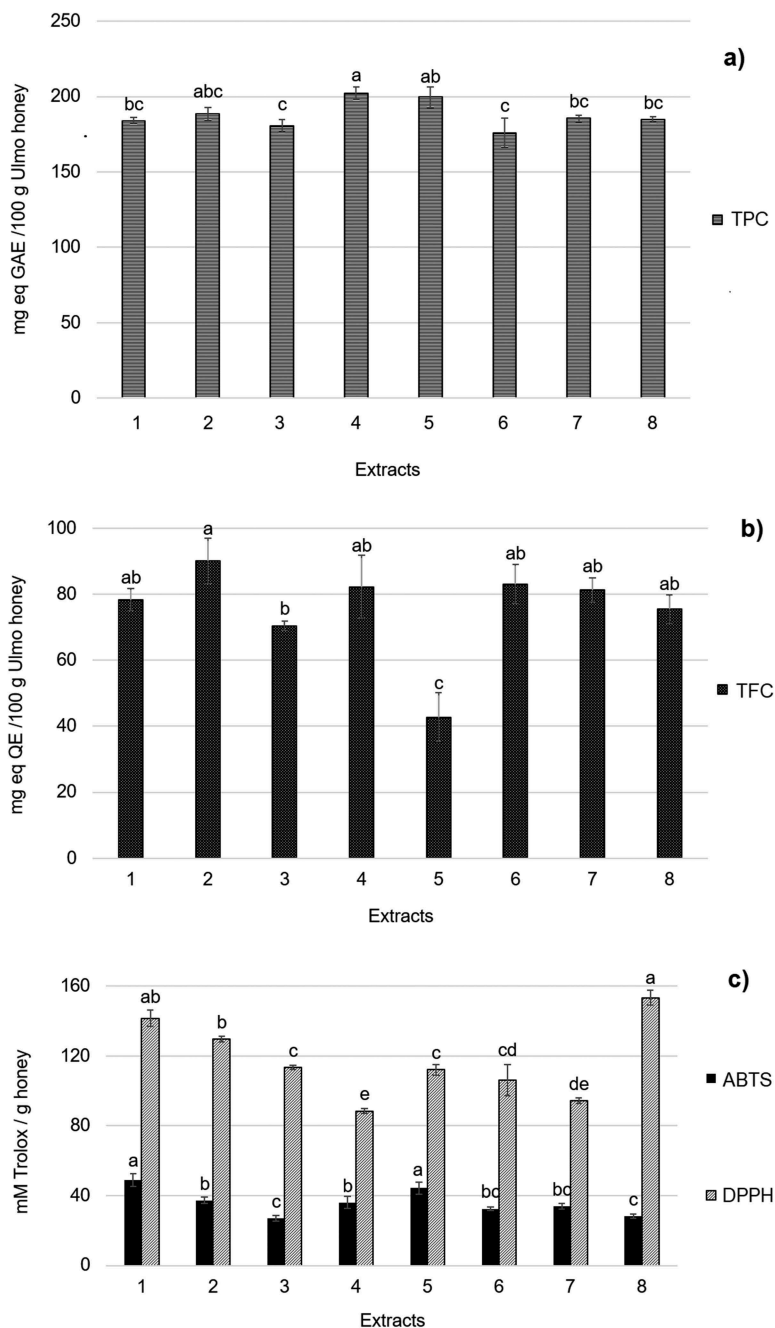


Figure 1. (a) Total phenolic content, (b) total flavonoid content, and (c) antioxidant capacities of Ulmo honey extracts evaluated using DPPH and ABTS radicals (Different letters indicate statistically significant differences between groups, $P < 0.05$).

Figura 1. (a) Contenido fenólico total, (b) contenido total de flavonoides y (c) capacidades antioxidantes de extractos de miel de Ulmo evaluados usando radicales DPPH y ABTS (letras diferentes indican diferencias estadísticamente significativas entre los grupos, $P < 0.05$).

a similar chemical profile, as shown in Table 2. Four phenolic acids (gallic, caffeic, coumaric, and chlorogenic acids), one isoprenoid (abscisic acid) and six flavonoids (pinocembrin, chrysin, quercetin, luteolin, apigenin, and rutin) were identified in the honey samples based on comparisons to the 22 available standards. The abscisic acid concentration was higher than those of the other compounds within all samples. Some of these compounds have been detected in other monofloral honeys. Gallic acid, myricetin, rutin, quercetin and naringenin were identified in *Quillaja saponiaria* (Quillay) honey from Central Chile (Montenegro et al., 2009), while kaempferol, quercetin, chrysin, syringic acid, and myricetin were detected in *Pinus* spp. honey from Greece (Karabagias, Badeka, Kontakos, Karabournioti, & Kontominas, 2014). *Erica* spp. honey samples from Portugal contained pinobanksin, pinocembrin, chrysin, galangin, *cis,trans-*

abscisic acid and *trans,trans-*abscisic acid (Ferrerres, Andrade, & Tomás-Barberán, 1996), indicating that the phenolic profile of honey would depend on its botanical origin, and also the isoprenoid abscisic acid, that has been used as floral marker also (Silva et al., 2013).

It is noteworthy that the polyphenols identified in the Ulmo honey samples are known to have biological activity. For example, it has been reported that gallic acid has antimicrobial, anticancer and antiviral activities (Khatkar, Nanda, Kumar, & Narasimhan, 2017), while rutin, also called vitamin P, chrysin and pinocembrin have demonstrated anti-inflammatory, anti-asthmatic, antitumor and antimicrobial activities. These health benefits are mainly attributed to the powerful antioxidant activities of these molecules as scavengers of free radicals (Hanieh et al., 2017; Mani & Natesan, 2018; Yang, Guo, & Yuan, 2008).

Table 2. Flavonoids and phenolic acids identified and quantified by HPLC MS-MS in Ulmo honey samples (mg/100 g honey).

	GalA	CinA	SyrA	FerA	ChIA	SinA	CafA	CouA	3HbA	Cat	Pin	Rut	Chr	Que	AbsA	Lut	VanA	Kae	Epi	Api	Res	Myr
1	0.24 ± 0.04ab	Nd	Nd	Nd	0.15 ± 0.03b	Nd	0.23 ± 0.02d	0.19 ± 0.04bc	Nd	Nd	1.48 ± 0.10d	Nd	0.26 ± 0.02c	0.17 ± 0.04b	6.35 ± 0.14abc	0.06 ± 0.01b	Nd	Nd	Nd	0.02 ± 0.00b	Nd	Nd
2	0.24 ± 0.05ab	Nd	Nd	Nd	Nd	Nd	0.27 ± 0.03cd	0.21 ± 0.02c	Nd	Nd	1.43 ± 0.12d	Nd	0.40 ± 0.04bc	0.19 ± 0.03b	13.03 ± 0.32cde	0.07 ± 0.01b	Nd	Nd	Nd	0.01 ± 0.00b	Nd	Nd
3	0.24 ± 0.05ab	Nd	Nd	Nd	Nd	Nd	0.14 ± 0.05bc	0.14 ± 0.04bc	Nd	Nd	0.61 ± 0.10bc	Nd	0.2 ± 0.08b	0.18 ± 0.04b	9.93 ± 0.17bc	0.05 ± 0.01ab	Nd	Nd	Nd	0.01 ± 0.00b	Nd	Nd
4	0.36 ± 0.04b	Nd	Nd	Nd	Nd	Nd	0.32 ± 0.04bcd	0.17 ± 0.03c	Nd	Nd	2.73 ± 0.23bcd	Nd	0.67 ± 0.04bc	0.20 ± 0.03b	2.99 ± 0.18de	0.06 ± 0.01b	Nd	Nd	Nd	0.03 ± 0.00ab	Nd	Nd
5	0.32 ± 0.06ab	Nd	Nd	Nd	0.21 ± 0.04a	Nd	0.44 ± 0.03cd	0.41 ± 0.06ab	Nd	Nd	1.86 ± 0.14cd	Nd	0.64 ± 0.03c	0.24 ± 0.05ab	17.76 ± 0.77a	0.10 ± 0.01b	Nd	Nd	Nd	0.03 ± 0.00ab	Nd	Nd
6	0.36 ± 0.07a	Nd	Nd	Nd	Nd	Nd	0.18 ± 0.06 ab	0.28 ± 0.03bc	Nd	Nd	1.56 ± 0.14a	Nd	0.52 ± 0.10a	0.21 ± 0.04b	16.68 ± 0.19e	0.06 ± 0.01b	Nd	Nd	Nd	0.02 ± 0.00a	Nd	Nd
7	0.32 ± 0.05ab	Nd	Nd	Nd	Nd	Nd	0.15 ± 0.03cd	0.29 ± 0.03bc	Nd	Nd	0.73 ± 0.13cd	Nd	0.17 ± 0.04bc	0.26 ± 0.07a	20.81 ± 0.14bcd	0.06 ± 0.01b	Nd	Nd	Nd	0.02 ± 0.00b	Nd	Nd
8	0.26 ± 0.06ab	Nd	Nd	Nd	Nd	Nd	0.44 ± 0.09a	0.21 ± 0.08a	Nd	Nd	1.86 ± 0.17b	Nd	0.64 ± 0.03a	0.24 ± 0.05ab	18.4 ± 0.17ab	0.10 ± 0.02a	Nd	Nd	Nd	0.02 ± 0.00a	Nd	Nd

Antiradical capacity against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS); Antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH); total phenolic content (TPC); total flavonoid content (TFC); gallic acid (GalA); Cinnamic acid (CinA); Syringic acid (SyrA); Ferulic acid (FerA); Sinapic acid (SinA); 3-Hydroxybenzoic acid (3HbA); Catechin (Cat); Chlorogenic acid (ChIA); Caffeic acid (CafA) Coumaric acid (CouA); Pinocembrin (Pin); Rutin (Rut); Chrysin (Chr); Quercetin (Que); Abscisic acid (AbsA); Luteolin (Lut); Vanillic acid (VanA); Kaempferol (Kae); Epicatechin (Epi); Apigenin (Api); Resveratrol (Res); Myricetin (Myr); Non detected (Nd). (Different letters indicate statistically significant differences between groups, $P < 0.05$).

Capacidad antiradical sobre 2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfónico) (ABTS); Actividad antiradical sobre 2,2-difenil-1-picrylhidrazil (DPPH); contenido fenólico total (TPC); contenido total de flavonoides (TFC); ácido gálico (GalA); Ácido cinámico (CinA); Ácido síringico (SyrA); Ácido ferúlico (FerA); Ácido sinápico (SinA); Ácido 3-hidroxibenzoico (3HbA); Catequina (Cat); Ácido clorogénico (ChIA); Ácido caféico (CafA) ácido cumárico (CouA); Pinocembrina (Pin); Rutina (Rut); Crisina (Chr); Quercetina (Que); Ácido abscísico (AbsA); Luteolina (Lut); Ácido vanílico (VanA); Kaempferol (Kae); Epicatequina (Epi); Apigenina (Api); Resveratrol (Res); Miricetina (Myr); No detectado (Nd). (Letras diferentes indican diferencias estadísticamente significativas entre los grupos, $P < 0.05$).

A correlation study was conducted between the polyphenolic composition and the antioxidant properties of the Ulmo honey samples using a Pearson multivariate analysis (Table 3). High positive correlations were found between the concentration of chlorogenic acid and the antioxidant activity of the samples as measured by the ABTS method (0.824) and between the antioxidant activity as measured by the DDPH assay and the nectar source Arrayán (0.844). There were no statistically significant correlations between ABTS/TPC, DPPH/TPC, TFC/DPPH, DPPH/ABTS and TFC/TPC. Similar conclusions were found in other monofloral honeys [34]. These results suggested that the antioxidant capacity of Ulmo honey is influenced by the content of some of the phenolics in the samples.

On the other hand, Principal Component Analysis (PCA) showed that there were no distinctive groups according to the difference in the contribution of the secondary species (Figure 2(a)). However, there was a relationship between Tineo and TP content, which implies that the highest concentration of Tineo generates the highest concentration of TP content. There is also an inverse correlation between TP content and Arrayán, with sourcing from Arrayán species generating a lower TP content. There were no strong relationships between the TF content and any of the other compounds, with the Ulmo, nor with the accompanying species (Figure 2(b)). In regards to the antioxidant activity, no direct relationships were observed between ABTS radical scavenging activity and either the botanical origin or the polyphenol content or molecules (Figure 2(c)). A direct relationship between the antioxidant activity, the Arrayán source of nectar, and the flavonoid luteolin was observed, as was one between the DPPH antioxidant activity and luteolin (Figure 2(d)).

3.3. Phenolic and flavonoid contents and antibacterial properties

The antibacterial activity of the eight Ulmo honey samples were tested in inhibition zone assays. Most of the honey samples inhibited growth of *S. aureus*, *St. pyogenes*, *Ps. aeruginosa* and *E. coli* (Figure 3). Samples 2, 4, 5 and 7 showed the greatest antibacterial activity against *S. aureus*. Samples 1 to 7 showed similar inhibition activity against *St. pyogenes*, while sample 8 showed slightly less activity. The highest activity against *E. coli* was observed in samples 1 and 8, both with Arrayán as secondary species of origin. Sample 5 exhibited the highest antibacterial activity against *Ps. aeruginosa*, while sample 2 showed the lowest. Of the microbes assayed, Ulmo honey samples showed greater inhibition of Gram-positive bacteria compared to Gram-negative bacteria.

Previous studies reveal similar inhibition patterns. Sherlock et al. (2010) reported that Ulmo honey had inhibitory activity against *E. coli*, *Ps. aeruginosa* and five strains of *S. aureus*. Acevedo et al. (2017) also reported activity against *Aeromonas hydrophilla*, *Klebsiella pneumoniae* and *Salmonella enterica*. Having a greater number of Ulmo honey samples from different geographical origins and harvest seasons could strengthen the relevance of *Eucryphia cordifolia* in the local beekeeping industry.

Correlation analysis (Table 3) showed statistically significant correlations between the inhibition of *Ps. aeruginosa* and chlorogenic acid levels (0.733) and between inhibition of *E. coli* and the percentage of Arrayán as secondary species

Table 3. Pearson correlation coefficients between botanical origin (percentage of Ulmo, Arrayán, Alfalfa chilota and Tineo); antioxidant characteristics (ABTS, DPPH, TPC, TFC); and specific phenolic acids and flavonoids presents in Ulmo honey samples.

Tabla 3. Coeficientes de correlación de Pearson entre origen botánico (porcentaje de Ulmo, Arrayán, Alfalfa chilota y Tineo); características antioxidantes (ABTS, DPPH, TPC, TFC); y ácidos fenólicos y flavonoides presentes en muestras de miel de Ulmo.

	Ulm	Arr	Alf	Tin	TPC	TFC	ABTS	DPPH	GaIA	ChiA	CafA	CouA	Pin	Chr	Que	AbsA	Lut	Api	Spy	Sau	Eco	Psa			
Ulm	1.000																								
Arr	-0.333 (0.420)	1.000																							
Alf	-0.557 (0.151)	-0.470 (0.240)	1.000																						
Tin	0.212 (0.614)	-0.226 (0.590)	0.297 (0.475)	1.000																					
TPC	0.136 (0.748)	-0.175 (0.678)	0.173 (0.682)	0.651 (0.081)	1.000																				
TFC	0.117 (0.783)	-0.157 (0.756)	0.190 (0.710)	0.339 (0.652)	0.395 (0.339)	1.000																			
ABTS	0.477 (0.233)	-0.192 (0.649)	0.003 (0.683)	0.003 (0.994)	0.395 (0.333)	0.278 (0.505)	1.000																		
DPPH	0.049 (0.908)	0.844 (0.009)	0.550 (0.158)	0.517 (0.433)	0.324 (0.802)	0.012 (0.977)	0.107 (0.802)	1.000																	
GaIA	-0.583 (0.130)	0.202 (0.631)	0.226 (0.591)	0.545 (0.163)	0.368 (0.370)	0.337 (0.414)	0.224 (0.594)	0.174 (0.680)	1.000																
ChiA	0.251 (0.549)	-0.118 (0.781)	-0.062 (0.885)	0.214 (0.610)	0.366 (0.373)	0.752 (0.031)	0.824 (0.012)	0.187 (0.657)	0.099 (0.815)	1.000															
CafA	-0.536 (0.171)	0.590 (0.124)	0.178 (0.674)	0.111 (0.793)	0.415 (0.307)	0.061 (0.886)	0.749 (0.032)	0.323 (0.436)	0.561 (0.148)	-0.470 (0.240)	1.000														
CouA	-0.503 (0.204)	0.754 (0.031)	0.207 (0.622)	0.292 (0.483)	0.005 (0.991)	0.505 (0.202)	0.179 (0.671)	0.621 (0.100)	0.490 (0.218)	0.240 (0.567)	0.637 (0.090)	1.000													
Pin	-0.339 (0.412)	0.145 (0.732)	0.078 (0.854)	0.018 (0.965)	0.497 (0.210)	0.154 (0.715)	0.618 (0.102)	0.025 (0.953)	0.634 (0.092)	0.454 (0.259)	0.823 (0.012)	0.219 (0.602)	1.000												
Chr	-0.498 (0.209)	0.403 (0.321)	0.057 (0.893)	0.221 (0.599)	0.594 (0.121)	0.160 (0.705)	0.721 (0.044)	0.210 (0.617)	0.688 (0.059)	0.938 (0.205)	0.938 (0.001)	0.428 (0.290)	0.428 (0.001)	1.000											
Que	-0.768 (0.026)	-0.015 (0.972)	0.861 (0.006)	0.454 (0.257)	0.110 (0.795)	0.233 (0.579)	0.100 (0.813)	0.155 (0.713)	0.351 (0.394)	0.043 (0.894)	0.043 (0.920)	0.186 (0.659)	0.182 (0.666)	-0.182 (0.958)	1.000										
AbsA	-0.170 (0.687)	0.421 (0.298)	-0.016 (0.969)	0.457 (0.255)	0.200 (0.635)	0.264 (0.527)	0.264 (0.527)	0.546 (0.162)	0.156 (0.713)	0.638 (0.089)	0.007 (0.987)	0.729 (0.040)	0.423 (0.296)	-0.212 (0.614)	0.331 (0.424)	1.000									
Lut	-0.705 (0.051)	0.663 (0.073)	0.016 (0.970)	0.251 (0.548)	0.237 (0.572)	0.176 (0.677)	0.707 (0.050)	0.378 (0.356)	0.814 (0.191)	0.877 (0.438)	0.004 (0.987)	0.014 (0.987)	0.497 (0.210)	0.722 (0.043)	0.278 (0.505)	0.369 (0.369)	1.000								
Api	-0.491 (0.217)	0.381 (0.351)	-0.222 (0.597)	0.114 (0.788)	0.046 (0.915)	0.121 (0.776)	0.307 (0.459)	0.109 (0.797)	0.710 (0.048)	0.114 (0.788)	0.757 (0.030)	0.532 (0.175)	0.795 (0.257)	0.763 (0.466)	-0.091 (0.831)	0.105 (0.804)	0.557 (0.151)	1.000							
Spy	0.641 (0.087)	-0.772 (0.024)	0.195 (0.643)	0.113 (0.790)	0.138 (0.744)	0.063 (0.882)	0.514 (0.193)	0.350 (0.396)	0.239 (0.569)	0.380 (0.353)	0.681 (0.063)	0.687 (0.060)	0.257 (0.539)	0.466 (0.244)	-0.168 (0.691)	0.239 (0.568)	0.816 (0.014)	-0.816 (0.168)	0.539 (0.307)	1.000					
Sau	-0.079 (0.853)	-0.692 (0.057)	0.621 (0.100)	0.236 (0.573)	0.444 (0.270)	-0.016 (0.969)	0.097 (0.819)	0.775 (0.024)	-0.059 (0.581)	-0.608 (0.110)	-0.608 (0.110)	-0.600 (0.116)	-0.432 (0.285)	-0.527 (0.179)	0.423 (0.297)	0.247 (0.555)	-0.434 (0.282)	-0.415 (0.307)	0.261 (0.533)	1.000					
Eco	-0.252 (0.547)	0.832 (0.010)	-0.319 (0.441)	0.290 (0.486)	0.368 (0.370)	0.109 (0.797)	0.042 (0.922)	0.786 (0.021)	0.147 (0.729)	0.436 (0.280)	0.436 (0.280)	0.635 (0.091)	0.124 (0.771)	0.314 (0.448)	0.042 (0.922)	0.383 (0.349)	0.433 (0.284)	0.268 (0.521)	-0.421 (0.299)	0.261 (0.533)	1.000				
Psa	0.006 (0.989)	-0.458 (0.253)	0.434 (0.283)	0.198 (0.638)	0.248 (0.554)	-0.866 (0.005)	0.370 (0.367)	0.255 (0.543)	0.108 (0.799)	0.733 (0.039)	-0.347 (0.400)	0.145 (0.732)	-0.306 (0.462)	-0.369 (0.369)	0.339 (0.412)	0.545 (0.163)	-0.132 (0.756)	-0.221 (0.599)	0.438 (0.278)	0.210 (0.618)	0.438 (0.598)	1.000			

Antiradical capacity against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS); Antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH); total phenolic content (TPC); total flavonoid content (TFC); Gallic acid (GaIA); Cinnamic acid (CinA); Syringic acid (SyrA); Ferulic acid (FerA); Sinapic acid (SinA); 3-Hydroxybenzoic acid (3HbA); Catechin (Cat); Chlorogenic acid (ChIA); Caffeic acid (CafA); Coumaric acid (CouA); Pinocembrin (Pin); Rutin (Rut); Chrysin (Chr); Quercetin (Que); Abscisic acid (AbsA); Luteolin (Lut); Vanillic acid (VanA); Kaempferol (Kae); Epicatechin (Epi); Apigenin (Api); Resveratrol (Res); Myricetin (Myr); *S. aureus* (Spy); *E. coli* (Eco); *Ps. aeruginosa* (Psa). (Different letters indicate statistically significant differences between groups, $P < 0.05$).

Capacidad antiradical sobre 2,2'-azino-bis (ácido 3-etilbenzotiazolina-6-sulfónico) (ABTS); Actividad antiradical sobre 2,2-difenil-1-picrylhydrazyl (DPPH); contenido total de flavonoides (TFC); ácido gálico (GaIA); Ácido cinámico (CinA); Ácido siringico (SyrA); Ácido ferúlico (FerA); Ácido sinápico (SinA); Ácido 3-hidroxibenzoico (3HbA); Catequina (Cat); Ácido clorogénico (ChIA); Ácido cafeico (CafA); Ácido cumárico (CouA); Pinocembrina (Pin); Rutina (Rut); Crisina (Chr); Quercetina (Que); Ácido abscísico (AbsA); Luteolina (Lut); Ácido vanílico (VanA); Kaempferol (Kae); Epicatequina (Epi); Apigenina (Api); Resveratrol (Res); Miricetina (Myr); No detectado (Nd). (Letras diferentes indican diferencias estadísticamente significativas entre los grupos, $P < 0.05$).

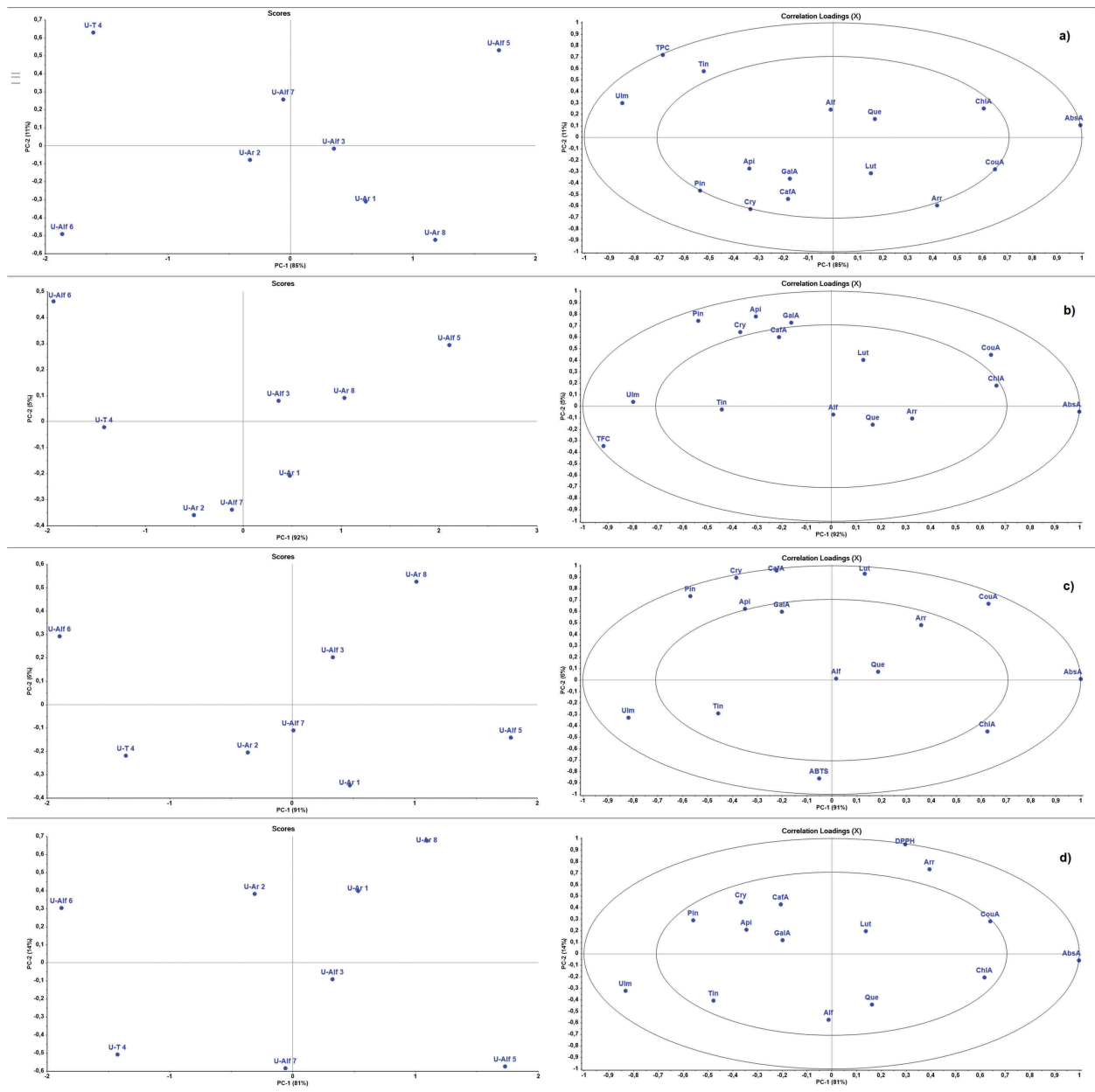


Figure 2. Score/loading correlation plots of Ulmo honey samples obtained through PCA for (a) total phenolic content (TPC), (b) total flavonoid content (TFC), (c) antioxidant capacity against 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), (d) antioxidant capacity against 2,2-diphenyl-1-picrylhydrazyl (DPPH). Gallic acid (GalA); Chlorogenic acid (ChIA); Caffeic acid (CafA); Coumaric acid (CouA); Pinocembrin (Pin); Chrysin (Chr); Quercetin (Que); Abscisic acid (AbsA); Luteolin (Lut); Apigenin (Api).

Figura 2. Gráficos de correlación de muestras de miel de Ulmo obtenidas a través de análisis de componentes principales para (a) contenido fenólico total (TPC), (b) contenido de flavonoides totales (TFC), (c) capacidad antioxidante sobre 2,2'-azino-bis (Ácido 3-etilbenzotiazolina-6-sulfónico) (ABTS), (d) capacidad antioxidante sobre 2,2-difenil-1-picrilhidrazilo (DPPH). Ácido gálico (GalA); Ácido clorogénico (ChIA); Ácido cafeico (CafA); Ácido cumárico (CouA); Pinocembrina (pin); Crisina (Chr); Quercetina (Que); Ácido abscísico (AbsA); Luteolina (Lut); Apigenina (Api).

(0.832). No correlation between antibacterial activity and botanical origin was observed, likely since the dominance of *Eucryphia cordifolia* is not significant different among the samples (range $92.2 \pm 3.9\%$, as shown in Table 1)

The PCA indicated that there were no distinctive groups according to the contribution of secondary species (Figure 4(a)). Despite this, relationships were established between the botanical origin, polyphenols and antibacterial activity (Figure 4(b)). The antibacterial activity against *Ps. aeruginosa* showed a direct relationship with alfalfa sourcing, Ulmo and chlorogenic acid, abscisic acid and quercetin, which are mostly linked to the alfalfa content. The antibacterial activity against *St. pyogenes* and *S. aureus* showed direct relationships with the amount of Ulmo and Tineo

sourced nectar. The inhibition of *Ps. aeruginosa* had a greater relationship with Tineo content than with Ulmo content. Both antibacterial activities showed indirect correlations with alfalfa sourcing, myricetin, quercetin and coumaric acid. The inhibition of *E. coli* was directly related to Arrayán sourcing and the presence of apigenin and chrysin, and to a lesser extent, with pinocembrin, caffeic acid and gallic acid.

4. Conclusions

In this study, several Ulmo honey samples were compared for phenolic composition, antioxidant and antimicrobial activities for the first time. The dominance of *Eucryphia cordifolia* in the samples range from 86.6% to 96.8%. Ulmo honey contained

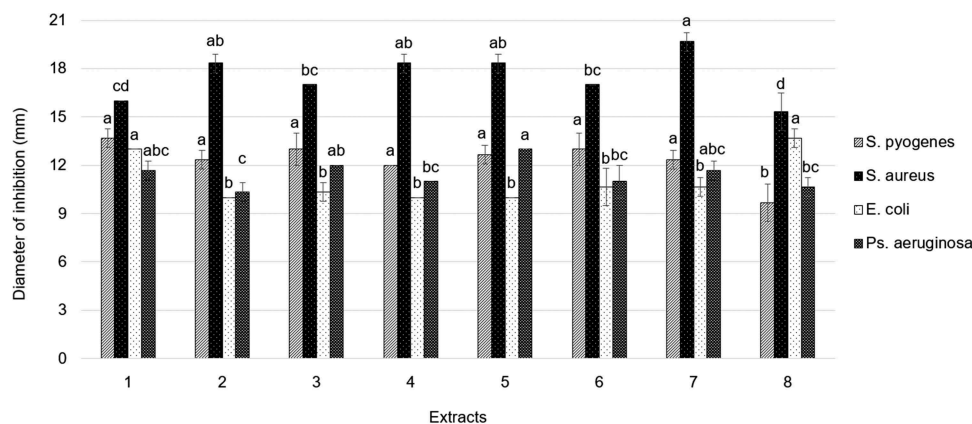


Figure 3. Antibacterial activity of extracts of Ulmo honey samples evaluated against *St. pyogenes*, *S. aureus*, *E. coli* and *Ps. aeruginosa* (Different letters indicate statistically significant differences between groups, $P < 0.05$).

Figura 3. Actividad antibacteriana de extractos de muestras de miel de Ulmo evaluadas sobre *St. pyogenes*, *S. aureus*, *E. coli* y *Ps. aeruginosa* (letras diferentes indican diferencias estadísticamente significativas entre los grupos, $P < 0.05$).

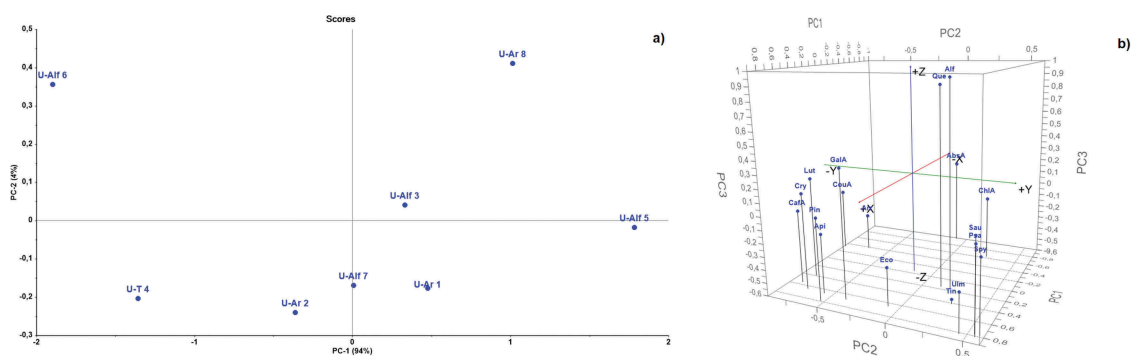


Figure 4. (a) Score plot of botanical origin and (b) 3D loading correlation plots of antibacterial activities, botanical origins and polyphenols. Arrayán (Arr); Alf (Alfalfa); Tineo (Tin); Ulm (Ulmo); Gallic acid (GalA); Chlorogenic acid (ChIA); Caffeic acid (CafA); Coumaric acid (CouA); Pinocembrin (Pin); Chrysin (Chr); Quercetin (Que); Abscisic acid (AbsA); Luteolin (Lut); Apigenin (Api); *S. pyogenes* (Spy); *S. aureus* (Sau); *E. coli* (Eco); *Ps. aeruginosa* (Psa).

Figura 4. (a) Gráfico de puntuación de origen botánico y (b) Gráficos de correlación, en 3 dimensiones, de la actividad antibacteriana, origen botánico y compuestos bioactivos de todas las muestras analizadas. Arrayán (Arr); Alf (Alfalfa); Tineo (estaño); Ulm (Ulmo); Ácido gálico (GalA); Ácido clorogénico (ChIA); Ácido cafeico (CafA); Ácido cumárico (CouA); Pinocembrina (pin); Crisina (Chr); Quercetina (Que); Ácido abscísico (AbsA); Luteolina (Lut); Apigenina (Api); *S. pyogenes* (espia); *S. aureus* (Sau); *E. coli* (Eco); *Ps. aeruginosa* (Psa).

gallic, caffeic, coumaric, abscisic and chlorogenic acids as well as pinocembrin, chrysin, quercetin, luteolin, apigenin, and rutin. These polyphenols content was contrast to the high antioxidant activity and strong antibacterial activity against *S. aureus*, *St. pyogenes*, *Ps. aeruginosa* and *E. coli*. According to PCA analysis, these activities could be related to the polyphenols present in the samples, especially chlorogenic acid. On the other hand, this study highlights how phenolic profiles are significant in the biological activity of honey samples and that further research should focus on identifying a broad set of molecules in a larger set of honey samples.

Disclosure statement

No potential conflict of interest was reported by the authors.

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