SHORT COMMUNICATION

Changes in the antibacterial capacity of Ulmo honey in relation to the contribution of *Eucryphia cordifolia* pollen

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Summary
Ulmo honey is known for its antibacterial characteristics. However, the importance of the content from *Eucryphia cordifolia* in the honey regarding its non-peroxidic antibacterial capacity is unknown. The present work evaluated the antibacterial activity of Ulmo honey with various percentages of pollen from *E. cordifolia* against various bacteria, determine the non-peroxidic capacity of Ulmo honey and, finally, compare that capacity with Manuka and Jarrah honeys. The antibacterial activity was evaluated by agar diffusion test and the non-peroxide capacity was evaluated by the reactivity with the catalase enzyme. The tests were carried out against *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus pyogenes*. The results showed a relationship between the percentage of floral pollen from *E. cordifolia* and the antibacterial activity of Ulmo honey. It was also observed than the antibacterial activity of Ulmo honey can have values similar to Manuka and Jarrah honeys.

Keywords
Eucryphia cordifolia; Eucalyptus marginate; Leptospermum scoparium; antimicrobial activity; catalase activity

The antibacterial activity of honey is closely related to its composition and the plant species used by bees to make it. The antibacterial capacity of honeys has been associated with its acidity, osmolarity, hydrogen peroxide and other, mainly phenolic, compounds from plants, broadly defining the origin in two types: peroxide and non-peroxide, being in most of the honeys mainly peroxide capacity [1]. Manuka honey is the best-known honey internationally. It is produced from the nectar of the flowers of the Manuka tree (*Leptospermum scoparium*), mainly in New Zealand and Australia. It was shown that the antibacterial activity of most honeys is due to the presence of hydrogen peroxide. However, in some honeys such as Manuka, this activity may be due to a non-peroxide component that is not identified, which is called "Unique Manuka Factor" (UMF) [2]. Mavric et al. [3] identified and quantified this UMF, which was recognized as a component called methylglyoxal, a compound to which its antibacterial activity has been attributed.

In Australia, another interesting honey is produced from Jarrah (*Eucalyptus marginata*) trees that only bloom once every two years. It is high in fructose and low in glucose and is characterized by a low glycemic index (*GI*) [4]. Its biocapacity is usually reported commercially through a value of total activity (*TA*). It is a dark and syrupy honey with a delicious caramel flavour, having high antibacterial activity that was found to be hydrogen peroxide-dependent [5].

Ulmo honey is produced from the nectar collected by bees from the flowers of the endemic Chilean tree *Eucryphia cordifolia* with the same name, Ulmo. Its antibacterial characteristics were found to be associated mainly with phenolic compounds (coumaric acid, ferulic acid, salicylic acid) and effects synergistic [6–8], however, no evaluation of non-peroxide capacity has been reported.

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There are no records of the evaluation of the influence of the *E. cordifolia* pollen on the antibacterial activity of Ulmo honey.

The objective of this work was to evaluate the antibacterial activity of Ulmo honeys with various percentages of pollen from *E. cordifolia*, to determine the non-peroxide capacity of Ulmo honey and to compare its antibacterial activity with Manuka and Jarrah honeys.

MATERIALS AND METHODS

Honey samples

Samples were purchased from local beekeepers from Southern Chile. The botanical origin of honeys was determined according to Chilean Standard Normative 2981 [9]. Briefly, 10 g of honey were diluted in 10 ml of distilled water, centrifuged and the sediment was re-suspended in 0.1 ml of distilled water. Pollen grains were observed under optical microscope and were identified using a palinoteque (Tab. 1). For comparison, Manuka honey UMF 5+ (Comvita, Paengaroa, New Zealand), Manuka honey UMF 15+ (New Zealand Honey, Wanaka, New Zealand) and Jarrah honey TA 10+ (Ausmiel, Harden, Australia) were purchased at local market in Santiago (Chile). A sample of each honey was used for the assay and all samples were stored in darkness at normal ambient temperature (25 °C).

Chemicals

All chemicals were of analytical grade and were obtained from Sigma Aldrich (St. Louis, Missouri, USA).

Antibacterial activity

Antibacterial capacity was evaluated through the agar diffusion method against *Escherichia coli* ATCC-25922, *Staphylococcus aureus* ATCC-25923 and *Streptococcus pyogenes* ISP 364-00. The strains were propagated on Mueller Hinton agar (Sigma Aldrich) at 35 °C for 24 h. Then, selected colonies were suspended in saline solution 9 $g \cdot l^{-1}$ at 10^6 $CFU \cdot ml^{-1}$ (corresponding to 0.5 McFarland standard; Becton-Dickinson, Franklin Lakes, New Jersey, USA). Strains suspensions were swabbed on Mueller Hinton agar in Petri dishes, 6 mm diameter holes were made in it and filled with 100 μl of each honey (Ulmo, Manuka or Jarrah). The plates were incubated at 35 °C for 24 h. Then, the inhibition diameter that appeared around each hole was measured and reported in millimeters [8].

Non-peroxide activity

For each honey analysed, two solutions were made at a concentration of 500 $mg \cdot ml^{-1}$: the first solution with catalase and the second without catalase, only with sterile distilled water. A solution (0.57 $mg \cdot ml^{-1}$) of C-40 catalase, 14 000 units per milligram of protein (Sigma Aldrich) was used to remove the hydrogen peroxide present in the samples of honey, transforming the compound into water and oxygen.

Agar diffusion test

The sensitivity of the bacteria to each of the honey samples was evaluated by the agar diffusion method. For this, 90 mm × 15 mm Petri dishes were used, which were filled with 25 ml of tryptic soy agar (Sigma Aldrich). Once the medium had solidified, the plates were seeded with a bacterial inoculum and then, each plate was perforated with a sterile 6 mm diameter punch. Subsequently, 100 μl of the honey solution with catalase (C^+) or without catalase (C^-) were added in each well and 30 min were waited until hydrogen peroxide to transformed into water and oxygen. This took

Tab. 1. Ulmo honey samples with different contribution of pollen from *Eucryphia cordifolia*.

Honey samples	*E. cordifolia* pollen contribution [%]	Secondary pollen contribution		Terciary pollen contribution		Classification*
		Species	[%]	Species	[%]	
1	91.8	*Lotus pedunculatus*	2.9	*Luma apiculata*	2.2	Monofloral
2	85.3	*Lotus pedunculatus*	7.7	*Luma apiculata*	3.6	Monofloral
3	82.1	*Weinmannia trichosperma*	5.6	*Lotus pedunculatus*	3.1	Monofloral
4	81.3	*Luma apiculata*	6.2	*Weinmannia trichosperma*	4.5	Monofloral
5	71.9	*Lotus pedunculatus*	11.7	*Luma apiculata*	2.3	Monofloral
6	61.3	*Weinmannia trichosperma*	22.9	*Luma apiculata*	6.0	Monofloral
7	51.2	*Lotus pedunculatus*	38.5	*Luma apiculata*	3.8	Monofloral
8	39.8	*Lotus pedunculatus*	19.4	*Azara petrolaris*	13.0	Polyfloral
9	30.2	*Weinmannia trichosperma*	26.5	*Lotus pedunculatus*	11.9	Polyfloral

* – classification according to Chilean Standard Normative 2981 [9].

place in the dark to avoid photodegradation of glucose oxidase. Finally, the plates were incubated at 37 °C for 24 h and the inhibition halo was measured in millimetres of three wells to obtain an average [10].

Statistical analysis

Variance was evaluated through Tukey's least significant difference (LCD) procedure at $p < 0.05$ using Statgraphics Centurion XVI (StatPoint-Technologies, Warrenton, Virginia, USA). All measurements were conducted in triplicate and reported as mean ± standard deviation.

RESULTS AND DISCUSSION

All Ulmo honey samples inhibited the growth of Gram-positive bacteria and these were more susceptible than Gram-negative bacteria (Fig. 1). It was observed that the zones of inhibition increased with the pollen percentage of the honeys, in the case of the Gram-positive bacteria *Staph. aureus* (12–20 mm) and *Str. pyogenes* (20–24 mm), while the Gram-negative *E. coli* was less sensitive (9–11 mm). Honey with 91.8 % and 71.9 % of *E. cordifolia* pollen had a greater effect by inhibiting the growth of *Staph. aureus* and *Str. pyogenes*, however, all honeys could be considered effective against *Str. pyogenes*. None of the honey samples was found to have an inhibitory effect against *E. coli*. This result revealed that the antibacterial activity varied according to the type of pathogen.

According to Abd-El Aal et al. [11], the antibacterial activity of honeys depends on the flowers from which the bees obtain the nectar. Fig. 1 shows the correlations between the percentage of *E. cordifolia* pollen and antibacterial activity, which may be related to the share of the predominant floral origin. In pollen, flavonoids are found almost exclusively as glycosides and, depending on their concentration and composition, a honey could have antimicrobial activity [12]. Moreover, honey without peroxide capacity is highly sought worldwide for its medicinal properties for human health and well-being. Removal of hydrogen peroxide capacity decreased the antimicrobial activity of several honey types against the test bacteria. The results on the capacity of hydrogen peroxide are shown in Tab. 2. The Gram-positive bacteria *Str. pyogenes* showed a mean inhibition diameter of 17.28 ± 3.68 mm, which is equivalent to an average decrease of 20 % in antimicrobial activity in the presence of catalase.

In particular, the honey sample 4 did not present a decrease in the inhibitory activity against *Str. pyogenes*, which can be attributed the non-hydrogen peroxide capacity. On the other hand, against *Staph. aureus* it presented a decrease of 28 %. Similar results were reported for Ulmo honey previously [13]. This loss of bacterial inhibition is mainly due to the elimination of peroxide capacity. However, since most of the samples of honey without peroxide cause inhibition, it indicates that they contain bioactive compounds,

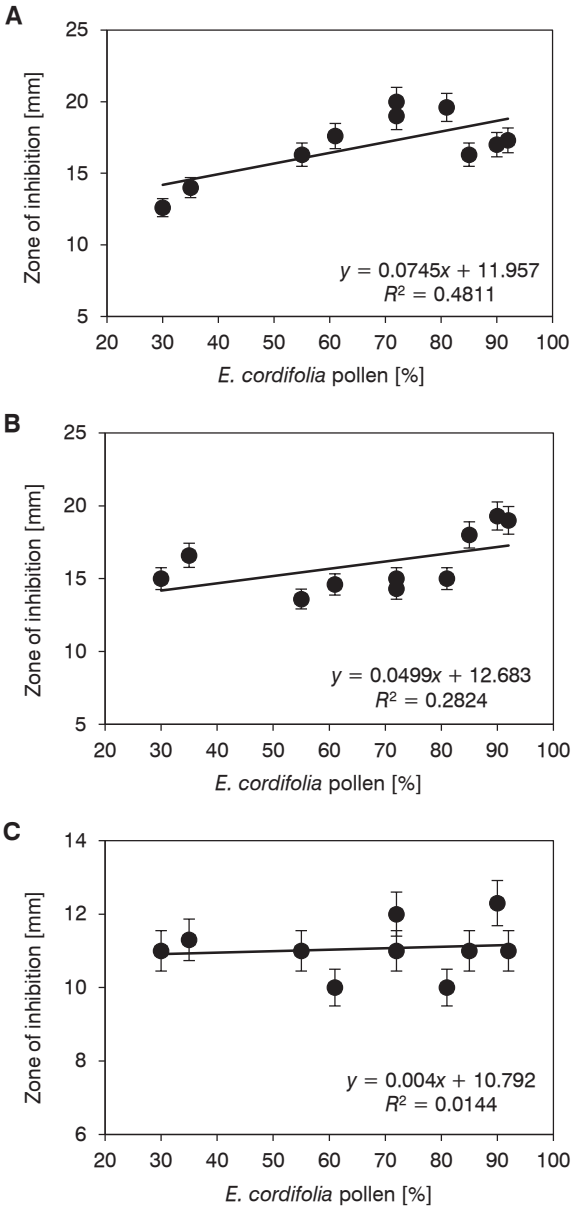

Fig. 1. Correlation between percentage of *E. cordifolia* pollen and antibacterial activity of Ulmo honeys.

A – *Staphylococcus aureus*, B – *Streptococcus pyogenes*, C – *Escherichia coli.*

Tab. 2. Non-peroxide activity of Ulmo honey samples.

Sample	Inhibition diameter [mm]					
	Staphylococcus aureus		*Streptococcus pyogenes*		*Escherichia coli*	
	C^-	C^+	C^-	C^+	C^-	C^+
1	17.0 ± 0.0	11.0 ± 0.0	24.0 ± 1.0	20.0 ± 0.0	12.5 ± 0.5	9.0 ± 0.0
2	20.0 ± 0.0	*	20.0 ± 0.0	15.0 ± 0.0	10.0 ± 0.0	*
3	17.0 ± 0.0	9.0 ± 0.0	20.0 ± 0.0	15.0 ± 0.0	11.0 ± 0.0	*
4	12.5 ± 0.5	9.0 ± 0.0	23.0 ± 0.0	23.0 ± 0.0	11.0 ± 0.0	*
5	17.0 ± 0.0	11.0 ± 0.0	24.0 ± 1.0	20.0 ± 0.0	12.5 ± 0.5	9.0 ± 0.0
6	20.0 ± 0.0	*	20.0 ± 0.0	15.0 ± 0.0	10.0 ± 0.0	*
7	15.0 ± 0.0	*	20.0 ± 0.0	13.0 ± 0.0	9.0 ± 0.0	*

Mean ± standard deviation is presented (n = 3). Values were not statistically different ($p < 0.05$).
* – no inhibition shown, C^+ – honey with catalase, C^- – honey without catalase.

Tab. 3. Comparison of antibacterial activity of Ulmo, Manuka and Jarrah honeys.

Sample	Inhibition diameter [mm]		
	Staphylococcus aureus	*Streptococcus pyogenes*	*Escherichia coli*
Jarrah AT 10+	20.0 ± 0.0^a	19.5 ± 0.5^b	10.5 ± 0.5^b
Manuka UMF 5+	17.0 ± 0.0^b	20.0 ± 0.5^b	10.5 ± 0.5^b
Manuka UMF 15+	19.5 ± 0.5^a	20.5 ± 0.0^a	15.0 ± 0.0^a
Ulmo	20.0 ± 0.0^a	21.5 ± 0.0^b	11.0 ± 0.0^b

Mean ± standard deviation is presented (n = 3). Different superscript letters indicate significant differences between groups ($p < 0.05$) according to Tukey's test.

possibly polyphenols that contribute to antibacterial activity. *E. cordifolia* belongs to the *Cunoniaceae* family, a family characterized by producing honeys with high antibacterial activity [10], and their polyphenols profile has been described [8].

Tab. 3 shows that Ulmo honey has comparable antibacterial activity with Manuka and Jarrah honeys ($p < 0.05$). On the other hand, only Manuka honey (UMF 15+) had an inhibitory effect against *E. coli* with a zone of inhibition value of 15 mm. These results are in agreement with reports published previously [12, 14, 15].

In general, the fact that Ulmo honey has antimicrobial activity in the absence of hydrogen peroxide indicates that it may be a possible therapeutic alternative against the resistant pathogenic microorganisms, however, further studies are necessary to understand the nature of the antibacterial activity of the honey.

CONCLUSIONS

This study reports, for the first time, the non-peroxide antibacterial activity of Ulmo honey. The results obtained show that Ulmo honey contains antibacterial compounds of non-peroxide character and the antibacterial activity is related to the content of *E. cordifolia* in the samples. Given its antibacterial activity, honey can be used as a natural antibiotic in nutraceutical, pharmaceutical or food formulations.

Acknowledgements

This work was supported by Comisión Nacional de Investigación Científica y Tecnológica (CONICYT) National Scholarship No. 21110822 (P.V.); Patricia Velasquez (PAI-CONICYT) doctoral thesis in the Industry Fellowship No. 781412002. Rafael Viteri is thankful to Vicerrectoría de Investigación of Pontificia Universidad Católica de Chile (VRI-UC, Santiago, Chile) scholarship.

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Received 20 April 2021; 1st revised 6 June 2021; 2nd revised 6 July 2021; accepted 13 July 2021; published online 9 September 2021.