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Chapter 3

The Value of Chilean Honey: Floral Origin Related to their Antioxidant and Antibacterial activities

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Additional information is available at the end of the chapter

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Abstract

Honey chemical composition is related to the plant species where nectar is collected by honeybees. Chilean beekeeping is characterized by a variety of honey types, some unique, due to a high participation of endemic and native species. In Chile, the most emblematic flower honey, both for its abundance and sensory characteristics, is ulmo honey (*Eucriphya cordifolia*) and quillay honey (*Quillaja saponaria*). Melissopalynological analyses are used to establish whether a honey is unifloral, where at least 45% or more pollen grains found in it belong to the same species. The antioxidant and antimicrobial activities of Chilean honey have been studied in the last years with excellent results. *Quillaja saponaria, Eucriphya cordifolia, Azara petiolaris*, and *Retanilla trinervia* are within the Chilean endemic species that produce unifloral honeys that show antioxidant potential and antibacterial activity against pathogenic gram positive and gram -negative bacteria and also multiresistant strains. These activities are mainly attributed to the phenolic compounds such as flavonoids. Among these attractive characteristics of honey, it is important to note that this product has low toxicity and the medicinal properties of honey will help to protect honeybees by adding value not only to the significantly important process of pollinating crops and native plants, but also for the medicinal importance of their products.

Keywords: Chile, *Apis mellifera*, honey, phenolic compounds, biological activity, antioxidant, antibacterial

1. Introduction

In Chile, the natural and endemic flora offers many plants with invaluable potential biological properties that may be inherited for products originated from this flora such as honeybee products. Likewise, beekeeping is active and there are several unifloral and endemic Chilean honey that have been reported to have important biological properties such as ulmo

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honey (originating from *Eucriphya cordifolia*), quillay honey (originating from *Quillaja saponaria*), tevo honey (originating from *Retanilla trinervis*), and others. The botanical origin of honey may be known through a quantitative and qualitative melissopalynological analysis. Honeybees are selective in the use of flower resources. The dominant plant community in Central Chile corresponds to the Matorral, an evergreen sclerophyllous vegetation with quillay and tebo as dominant plant species. The deep south of the country is dominated by temperate forest where ulmo is one of the dominant species. Biodiversity varies along an altitudinal or latitudinal gradient in Chile, so the beekeepers usually maintain their beehives along the native plant communities, so the bee products, as well as their potential biological properties will also be different depending on the botanical and geographical origin. Among the bioactive molecules inherited from a specific floral source, phenolic compounds obtained from honey have been related with the antioxidant and antibacterial properties that they show. Honey as a natural product offers many advantages that classify it as an excellent source of active molecules, which could be used as a treatment of human diseases in the forthcoming years. Among these attractive characteristics of honey, it is important to note that this product has low toxicity and the medicinal properties of honey will help to protect honeybees from disappearance by adding value not only to the significantly important process of pollinating crops and native plants, but also for the medicinal importance of their products.

2. Chile's unique geographical features and its endemic flora

The continental Chilean territory has an area close to 75 million hectares and is situated on the southwest border of South America. It has a length of approximately 4300 km from north to south and the average width is 180 km. Pits, terraces, mountainous regions, and valleys form Chile's diverse geomorphology, which together with the biogeographical isolation of a territory limited by geographical and climatic barriers, has configured a biodiversity characterized by a high level of endemism in ecosystems. Chile's vascular flora contains approximately between 5500 and 6000 species, without including subspecies and varieties. Although the number of species, compared with other South American countries, is not especially high, the most prominent trait of Chilean vascular flora is the presence of close to 50% endemic plant species, which gives the Chilean vascular flora a marked uniqueness [1]. Chilean apicultural production is defined by a high variety of honey types which contain a high percentage of nectar obtained from native plant species. The portion of nectar originating from native plants related to the endemism of Chilean flora result in the production of honey with unique characteristics.

The production of native and endemic monofloral honey is segregated into two large geographical areas: the first area corresponds to the central zone of Chile and the second corresponds to a region with a climatic transition from humid Mediterranean (VIII Region) to temperate humid (X Region). The central zone of Chile is of the five regions in the world that has a Mediterranean climate. It is characterized by a high level of endemism and biodiversity. Matorral is the dominant vegetal community in this zone. Characteristic matorral species include *Baccharis concave* (chilca), *Peumus boldus* (boldo), *Lithraea caustica* (litre),

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Trevoa trinervis (tevo), and *Q. saponaria* (soapbark tree). The central zone is characterized by the production of endemic monofloral honey from the quillay (*Q. saponaria*) and corontillo (*Escallonia pulverulenta*) species, while the southern zone, characterized by temperate forests, is characterized by native unifloral honey made from avellano *(Gevuina avellana)*, ulmo *(E. cordifolia)*, and tineo *(Weinmannia trichosperma)* [2].

3. Botanical origin of honey

Honeybees show great selectivity in the use of the vegetation surrounding their beehives. It has been shown that bees select plants with a high production of nectar, high concentration of sugar and that do not contain toxic compounds like certain alkaloids. Nevertheless, the presence of other secondary metabolites including terpenoids, phenolic acids, and flavonoids confer to honey import medicinal properties [3]. Nectar is an aqueous plant secretion whose content is mainly sugars and amino acids. It is collected by bees, particularly *Apis mellifera* L., and is converted into honey by enzymatic actions and dehydration, producing about 18% water content [4]. Honey is a food that contains about 200 substances and consists mainly of sugars, water, and other substances such as proteins (enzymes), organic acids, vitamins (especially vitamin B6, thiamine, niacin, riboflavin, and pantothenic acid), minerals (including calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, and zinc), pigments, solid particles derived from honey harvesting, a large variety of volatile compounds, and also secondary metabolites characteristic of the origin species like phenolic compounds and terpenes [4, 5].

Melissopalynology is the division of palynology, which studies the botanical and geographical origin of honey by subjecting honey sediment, and therefore pollen grain and the other structures therein, to microscopic analysis (**Figure 1**). Honey pollen profiles indicate floral diversity, forest vegetation, and species composition of plants that honeybees forage. The relative pollen frequency is utilized for tagging purposes and to ensure geographical origin, factors which considerably influence honey's commercial value. Furthermore, relative pollen frequency is also utilized as a traceability tool by food control institutions and to assess correlations with *in situ* climatic parameters such as rainfall and temperature, important external factors influencing pollinators and pollination networks [6, 7]. In Chile, the official policy (NCh2981.Of2005) established by the Standards Division of the National Institute for Standardization [2] indicates that the melissopalynological test must be used to differentiate the botanical origin of honey produced in this country. In agreement with this regulation, honey can be classified according to three types of botanical origins: monofloral, bifloral, or polyfloral. Monofloral or unifloral honeys are those where at least 45% or more pollen grains found in it belong to the same species; bifloral honeys are those where pollens from two species are dominant within the total pollen grains, so that, as a whole, both species cover more than 50% of the total pollen grains, and there is not a difference higher than 5% among them; and finally, polyfloral honeys are those where none of the requirements for monofloral and bifloral honeys are met, that is, those where no species reaches at least 45% of the total pollen grains, nor two of them covers more than 50% of the said total.

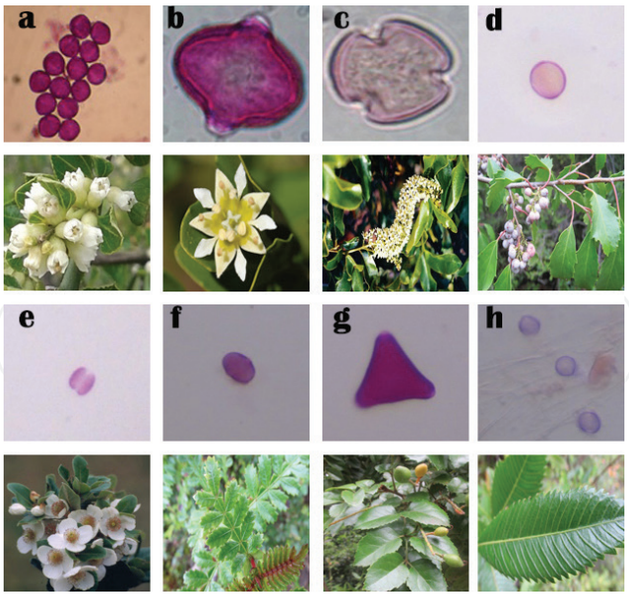


Figure 1. Plants species and respective pollens (microscope 400x) (a) *Retanilla trinervia* (tevo), (b) *Quillaja saponaria* (quillay), (c) *Escallonia pulverulenta* (corontillo), (d) *Azara petiolaris* (corcolén), (e) *Eucryphia cordifolia* (ulmo), (f) *Weinmannia trichosperma* (tineo), (g) *Gevuina avellana* (avellano chileno), and (h) *Caldcluvia paniculata* (tiaca).

The melissopalynology technique is quite laborious, time-consuming and requires a high-skilled and trained technician. Thus, a large number of research groups worldwide have focused their attention and studies on improving the knowledge of honey characterization. The most promising approach appears to be the simultaneous detection of multiple components utilizing spectroscopic methods along with statistical analysis. Chemometrics along with Raman, FTIR, NMR, and NIR spectroscopic methods have been used for defining the floral origin of honey and development of classification models. These procedures promptly provide quantitative information without complex pretreatment of samples and primarily through a single spectroscopic technique [6, 8, 9]. Despite advances in these methods in the last few years, limitations still exist in these studies related to the small number of samples and the validity of the proposed methods are rarely demonstrated [8]. Notwithstanding, the emerging new methods are making way to new frontiers in honey characterization. The most promising strategy appears to be the multidisciplinary one, which focuses on the detection of multiple components assisted by chemometrics. Apicultural industries and small producers will make the most of the advantages of more advanced methods which allow for more scrupulous controls, increasing the quality level and safety of honey and derivatives [9].

4. Chilean unifloral honey

Chile produces a limited number of unifloral honeys with native plant origins. Montenegro et al. [10] identified the species of native plants that *A. mellifera* uses as the most intensive

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source of nectar. These species include *Q. saponaria* (quillay, soapbark), *E. cordifolia* (ulmo), *G. avellana* (avellano), *E. pulverulenta* (corontillo), *R. trinervia* (tevo), *Caldcluvia paniculata* (tiaca), *W. trichosperma* (tineo), and species of genus *Azara* (corcólen). They are used as the source for monofloral honey, which are selected by honeybees mainly due to the volume and chemical composition of nectar offered by the flowers [4, 7, 10] (**Table 1**).

Origin	Common name	Plant species	Family
Zone			
Forest temperate (Southern Chile)	ulmo	*Eucryphia cordifolia* Cav.	Cunoniaceae
	tiaca	*Caldcluvia paniculata* (Cav.) D. Don	Cunoniaceae
	tineo	*Weinmannia trichosperma* Cav.	Cunoniaceae
	avellano	*Gevuina avellana* Molina	Proteaceae
Matorral (Central Chile)	quillay	*Quillaja saponaria* Monlina	Quillajaceae
	tevo	*Retanilla trinervia* (Gillies & Hook.) Hook. & Arn.	Rhamnaceae
	corontillo	*Escallonia pulverulenta* (Ruiz & Pav.) Pers.	Escalloniaceae
	corcolen	*Azara petiolaris* (D. Don) I.M. Johnst.	Salicaceae

Table 1. Botanical origin of unifloral honey in Chile.

A recent study of the biological properties of Chilean unifloral honeys indicates that Chilean native honey presented significant differences in their antioxidant as well as biological activity, which depends on the botanical and geographical origin, and can be associated with polyphenol content. Moreover, the presence of other species in the total botanical content of honey plays an important role in the modulation of its biological properties [11].

In Chile, the most emblematic flower honey, both for its abundance and sensory characteristics, is quillay (*Q. saponaria)* and ulmo (*E. cordifolia*). The antioxidant and antimicrobial activities of Chilean honey have been studied in the last years with excellent results. *Q. saponaria*, *E. cordifolia*, and *R. trinervia* are within the Chilean endemic species that produce monofloral honey that show antibacterial activity against pathogenic Gram-positive and Gram-negative bacteria and also multiresistant strains [4, 10–14]. With regard to antioxidant activity, honey from *Q. saponaria* and *Azara petiolaris* stand out due to potential shown in various *in vitro* models utilized to evaluate natural antioxidant capacity to inactivate reactive species. The positive correlation between phenolic compounds and antioxidant capacity is verified in some of these models. Phenolic compounds such as aromatic acids and flavonoids are considered to be responsible for antioxidant capacity since they have a chemical structure particularly suitable to exert an antioxidant action acting as free radical scavengers neutralizing reactive oxygen species and chelating metal ions.

5. Phenolic compounds

Phenolic compounds are plant-derived secondary metabolites, biosynthesized mainly for protection against stress and oxidative damage and transferred via the nectar to the honey. The intensity of the color of a honey may be associated with the antioxidant strength of the honey. The phenolic, flavonoid, and carotenoid content is increased in darker honeys and reduced in lighter more transparent honey. As a result, biological properties, such as antioxidant activities and antibacterial capabilities, of the honey are related to its color, and darker honey tends to have enhanced properties [4, 15].

The most common phenolic compounds are phenolic acids and flavonoids [16]. Phenolic acids constitute an important class of phenolic compounds with bioactive functions typically found in vegetable products and foods. Also are secondary metabolites required for normal operation of naturally occurring plants. They can be divided into two subgroups according to their structure: the hydroxybenzoic and hydroxycinnamic acids. Acids derived from hydroxybenzoic acids include þ-hydroxybenzoic, vanillic, syringic, salicylic (2-hydroxybenzoate), gallic, and ellagic. These compounds might be existing in soluble form in cells, along with sugars or organic acids, or formed with cells linked to lignins. Hydroxycinnamic acids occur normally in their conjugated form as esters of hydroxy acids such as tartaric acid and shikimic as well as in their pure form, including þ-coumaric, caffeic, ferulic, and sinapic acids. Flavonoids (flavones, flavonols, flavanones, flavanols, anthocyanidin, isoflavones and chalcones) are the largest group of plant phenolic compounds. This group represents over 50% of all naturally occurring phenolic compounds. They are generally distributed in the seeds, bark, leaves, and flowers of plants and trees. In plants, these compounds give protection, against pathogens, herbivores, and UV radiation [5, 17].

The qualitative and quantitative difference in the phenolic profile of honey according to the different botanical sources represents the scientific basis of the two main lines of research about the study of honey phenolic fraction. The first approach is focused on the evaluation of the bioactive properties of honeys while the second approach attempts to attribute the botanical and/or the geographical origin of honey based on the existence and the abundance of at least one or more specific phenolic compounds, thus proposed as chemical marker(s) of origin. The results of these research studies are relevant in both directions; honey of varying botanical origins show a wide range of health-promoting properties like antibacterial, antiinflammatory, antioxidant, and radical-scavenging activity [4, 16, 18]. A wide range of phenolic constituents are present in honey such as quercetin, caffeic acid, caffeic acid phenethyl ester (CAPE), acacetin, kaempferol, galangin, chrysin, pinocembrin, pinobanksin, and apigenin, which have promising effects in the treatment of some diseases [19, 20].

Pinocembrin, pinobanksin, and chrysin are the characteristic flavonoids of propolis and these flavonoids have been found in European [19, 21] and Chilean honey samples [22]. Small amounts of propolis might be incorporated into honey; therefore, propolis flavonoids might contribute to the phenolic composition of honey. In temperate areas, the main sources of propolis are poplar (*Populus*) bud exudates. The identification of propolis-derived compounds like pinocembrin and chrysin could have an important contribution to the phenolic composition and antioxidant activity in corcolen (*Azara petiolaris*) Chilean honey [22].

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Antioxidant, antiinflammatory, antimicrobial, antiviral, and antiulcerous action, and the capability for regulating enzymatic browning are some of the principal characteristics of honey primarily attributed to phenolic compounds. The implementation of honey polyphenols has recently gained great interest from the functional food, nutraceutical and pharmaceutical industries. However, the efficacy of polyphenols relies on the preservation of their stability and bioactivity. Phenolic compounds, as well as other organic compounds, are degraded depending on the environmental conditions to which they are subjected. Spanish honey samples were subjected to liquefaction and liquefaction/pasteurization and the phenolic compounds evaluated as to the impact of industrial heat treatment. Phenolic compounds found in these honey samples were caffeic and þ-coumaric acids and flavonoids naringenin, hesperetin, pinocembrin, chrysin, galangin, quercetin, and kaempferol. A significant decrease in the concentration of galangin, kaempferol, myricetin, and þ-coumaric acid was observed after heat treatment [23]. Moreover, some flavonoid glycosides present in honey demonstrate certain instability under slight alkaline conditions and high sensitivity to oxidation in the presence of slight oxidizing agents such as hydrogen peroxide, which is present in honey and is responsible for the degradation verified in the flavonoids analyzed [24, 25].

The complexity of a food matrix like honey implies that the target analytes are usually present in low concentrations, and this demands the adoption of a multistep analytical procedure able to provide a careful measurement of these quantities [16]. Procedures using Amberlite XAD-2 columns for cleaning the complex matrices of honey and isolation of their phenols are often performed. In some cases, this step would reduce the need for sample manipulation and give a sample extract uniformly enriched in all components of interest and free from interfering matrix components. In these procedures, aqueous- acidified honey solutions are passed through the columns to retain phenols in sorbent beds and afterward eluded with methanol [19, 26]. These extracts are widely employed in analytical methods, biological assays, and functional food development, since the presence of sugars gives the entire honey a syrupy texture, which causes difficulties for some analysis and preparations. However, recoveries of phenolic acids and flavonoids extracted from deionized water (pH 2) using Amberlite XAD-2 demonstrated different recovery percentages, probably depending on the structure of the phenols studied. Kaempferol, *p*-coumaric acid, and syringic acid were completely adsorbed, but the recovery of gallic acid, caffeic acid, and quercetin by methanol is much less efficient [13, 27–29].

6. Abscisic acid

Abscisic acid, a plant hormone related to the protection of plants in environmental stress conditions, has been detected in corcolen and quillay honeys. The existence of abscisic acid in nectar is well established and is affected by environmental conditions, which might regulate the biosynthesis of certain secondary metabolites, such as phenolic compounds and abscisic acid. The biosynthesis of these compounds may be stimulated by plants, lowering damages through their capacity to capture free radicals under stress conditions, and reduce the penetration of UV-B ultraviolet radiation. The representation of these compounds in honey produced from *Q. saponaria* may be associated with the high interannual variability of climate conditions of the central zone of Chile [13, 30].

7. Biological activities

Clinical investigations of the therapeutic potential of honey are gradually growing and scientific evidence for the efficacy of honey in some conditions is beginning to emerge. The healing effect of honey could be classified by its antiinflammatory, antibacterial, and antioxidant properties of its components. Furthermore, honey has been reported to be effective in gastrointestinal disorders, in healing of wounds and burns, and in treating venous ulcers [31].

8. Antioxidant activity

Over many years, honey from different parts of the world have been shown to be one of the highest potential natural products in which phenolics, flavonoids, ascorbic acids, and some enzymes serve as potent antioxidants [32]. The antioxidant properties of honey are derived from both enzymatic (e.g., catalase, glucose oxidase, and peroxidase) and nonenzymatic substances (e.g., phenolic compounds, ascorbic acid, α-tocopherol, carotenoids, amino acids, proteins, and Maillard reaction products). The quantity and kind of these antioxidants are mainly dependent on the floral source. The main functional components of honey are flavonoids. They contribute significantly to the total antioxidant activity of honey and they act by several mechanisms including direct trapping of reactive oxygen species, inhibition of enzymes responsible for producing superoxide anions, chelation of transition metals involved in processes forming radicals, and prevention of the peroxidation process by reducing alkoxyl and peroxyl radicals [5, 15, 29]. The antioxidant activity of flavonoids in the majority of cases relies on the number and position of hydroxyl groups, additional substituents, and the glycosylation of flavonoid molecules. The presence of specific hydroxyl groups in the flavonoid rings improves antioxidant activity. Substitution patterns in the A ring and B ring, and the 2,3-double bond (unsaturated) and 4-oxo group in the C ring affect the antioxidant action of flavonoids as well. The glycosylation of flavonoids reduces their antioxidant activity compared to the analogous aglycones [5, 33].

These antioxidants may help to protect cellular damages from oxidative stress and lower the risk of chronic diseases. Furthermore, in recent years, there has been an increase in new methods for the research of free radicals and antioxidants in relation with advances in human health. Various studies have demonstrated that neuronal and behavioral changes occur with ageing, including in the absence of degenerative disease. Current studies indicate that dietary intake of antioxidant nutrients and cognition is closely related. Evidence from epidemiological, experimental and clinical studies demonstrates that the consumption of foods with high levels of dietary antioxidants might prevent or lower the risk of cognitive deterioration [34]. Many research models have been established in chemical and/or biological systems for the studies of mechanisms of action of antioxidants. Generally, antioxidant ability was measured and presented as total antioxidant capacity (TAC) [35, 36], total antioxidant potentials (TRAP) [37, 38], Trolox equivalent antioxidant capacity (TEAC) [39], ferric reducing/antioxidant power (FRAP) [40], and oxygen radical absorption capacity (ORAC) [41]. Mechanistically, these methods are based on either a single-electron transfer reaction or a hydrogen atom

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transfer reaction from an antioxidant or oxidant to a free radical. The total antioxidant activity is related to the radical scavenging ability and reductive activity [42].

Montenegro et al. [11] studied the antioxidant activity of unifloral honeys (quillay, ulmo, avellana, tiaca) of native plants from Chile. In this study, was observed an important correlation between total phenolic content and antioxidant activity evaluated by ferric reducing activity power—FRAP method. The ferric reducing activity power assay directly measures antioxidants with a reduction potential below the reduction potential of the Fe^{3+}/Fe^{2+} couple and the reaction is reproducible and linearly related to the molar concentration of the antioxidant(s) present in the sample. Furthermore, some variations in antioxidant activity between honey samples with the same botanical origin were observed. This variability could be explained by different accompanying species and geographical origin zone of the honey [11].

The scavenging activity towards peroxyl/alkoxyl radicals (ORAC method) is one of the most employed assays. In fact, in the last years databases of the ORAC index of foods have been built to emphasize the benefits of establishing the antioxidant capacity of polyphenol-rich foods [43]. The method is based on the ability of antioxidants to prevent the consumption of a target molecule mediated by free radicals generated during the aerobic thermal decomposition of AAPH (2,2′-azo-bis(2-amidinopropane). The target molecules are most commonly used are beta-phycoerythrin, fluorescein, and pyrogallol red. The use of the pyrogallol red (PGR) as probe is related to the amount and reactivity of a given phenolic compound towards the free radicals generated in the AAPH (2,2′-azo-bis(2-amidinopropane) dihydrochloride) thermolysis. The ORAC-PGR index can be considered as a measure of the capacity of the sample to remove peroxyl and alkoxyl radicals [44]. In complex mixtures, concentration, chemical nature, and possibly the interaction between the antioxidants present in the sample determine this index.

Recently obtained results from our research group demonstrated that quillay honey's ORAC-PGR index is not correlated to phenolic compound content present in samples but is highly correlated to flavonoid content. This is due to the fact that flavonoids are the polyphenolic species to which the highest reactivity is attributed [45]. In addition, the ORAC-PGR index in honey of *A. petiolaris*, named commonly as corcolen honey, collected from honeybee colonies of the central zone of Chile was evaluated. The value of this parameter for these samples is correlated to the percentage of corcolen pollen present, meaning that mostly the phenolic compounds and compounds with scavenger capacity belong to corcolen species. These correlations are quite interesting since just by means of the melissopalynological assay there could be evidence of the phenolics composition as well as the antioxidant capacity of monofloral *Azara sp*. honey [22].

Finally, more recently, our group has obtained results indicating that honey quillay compounds are reactive toward hypochlorite (HOCl). Hypochlorite has an important role in defense mechanisms that take part in the immune response toward microorganisms. However, it has also documented that hypochlorite, in certain pathophysiological conditions, can damage macromolecules including proteins, DNA, RNA, and cell membrane lipids, changing their biological function. The consumption of PGR-induced by hypochlorite is inhibited by compounds able to react with this reactive species. PGR-hypochlorite indexes obtained for quillay honey samples indicated high hypochlorite-mediated oxidation protection potential, these results being comparable to those obtained via Trolox—water-soluble vitamin E analogue (unpublished results).

9. Antibacterial activity

The broad-spectrum of antimicrobial activity of honey was demonstrated in various studies and reportedly exerts both bacteriostatic and bactericidal activities. The antimicrobial nature of honey depends on different factors acting singularly or synergistically, the most significant of which are phenolic compounds, pH of honey, H_2O_2, wound pH, and osmotic pressure exerted by the honey itself [15, 46]. The antibacterial capabilities of different unifloral Chilean honey, including ulmo honey (*E. cordifolia*), quillay honey (*Q. saponaria*), avellano honey (*G. avellana*), and tiaca honey (*C. paniculata*) were analyzed (**Table 2**). The methanolic extract of these honeys, obtained using Amberlite XAD-2 column, demonstrated better antibacterial capabilities than the honeys themselves, indicating an important role of the phenolic compounds in this activity. In *in vitro* assays, all of the honey extracts were able to inhibit the growth of *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*, and *Streptococcus pyogenes* determining minimal bactericidal concentration [4, 11].

Floral Origin	Antioxidant/Antibacterial activities	Reference
Quillay honey (phenolic extracts)	Antibacterial activity against *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus typhi, S. aureus, Streptococcus pneumoniae, Vibri cholerae* and antifungal activity against *Candida albicans*.	Montenegro et al. [11]
Quillay honey (entire honey and phenolic extracts)	Oxygen radical absorbance capacity (ORAC-PGR) index related to the capacity of the sample to remove peroxyl and alkoxyl radicals	Bridi et al. [45]
Ulmo honey	Comparison of the antimicrobial activity of ulmo and manuka honey against methicillin-resistant *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*.	Sherlock, et al. [14]
Ulmo honey (phenolic extracts)	Inhibition of *in vitro* growth of human pathogenic bacteria *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and fungicidal activity on fungi genera *Mucor, Rhizopus, Aspergillus, Candida* and *Penicillium*	Montenegro and Ortega [48].
Ulmo honey	Ulmo honey topical application supplemented with ascorbic acid improves regeneration in burns in guinea pig.	Schencke, et al. [49–51]
Ulmo honey	Clinical trial: topical treatment using ulmo honey associated with oral ascorbic acid showed excellent clinical results for the healing of venous ulcers	Calderon et al. [52]
Ulmo, quillay avellana and tiaca honeys	Ferric reducing antioxidant power	Montenegro et al. [11]
Corcolen honey	Oxygen radical absorbance capacity (ORAC-PGR), index related to the capacity of the sample to remove peroxyl and alkoxyl radicals	Giordano et al. [22]

Table 2. Review of antioxidant and antibacterial activity in unifloral Chilean honey.

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The manuka honey derived from the manuka tree (*Leptospermum scoparium)*, which grows as a shrub, or a small tree throughout New Zealand and eastern Australia is the best known of the honeys. It has been reported to have an inhibitory effect on around sixty species of bacteria, including aerobes and anaerobes, positives, and Gram-negatives. The antimicrobial activity exhibited against pathogenic bacteria such as *S. aureus* make this honey a promising functional food for the treatment of wounds. The potential of honey to assist with wound healing has been demonstrated repeatedly and the healing properties can be ascribed to the fact that it offers antibacterial activity, maintains a moist wound environment that promotes healing, and has a high viscosity that helps to provide a protective barrier to prevent infection [47]. A study compared the antimicrobial activity of the ulmo honey with manuka honey against five strains of methicillin-resistant *S. aureus, E. coli*, and *P. aeruginosa*. The ulmo honey had greater antibacterial activity against all methicillin-resistant *S. aureus* isolates tested than manuka honey and similar activity against *E. coli* and *P. aeruginosa* using agar diffusion assay. The minimum inhibitory concentration assay showed that a lower minimum inhibitory concentration was observed with ulmo honey than with manuka honey for all five methicillin-resistant *S. aureus* isolates. For the *E. coli* and *Pseudomonas* strains, equivalent minimum inhibitory concentration was observed. Due to its high antimicrobial activity, ulmo honey may warrant further investigation as a possible alternative therapy for wound healing [14]. In Chile, ulmo honey extract has been patented for its bactericidal and fungicidal properties [48]. The document relates to uses of an extract of unifloral ulmo honey, rich in phenolic compounds, able to inhibit the *in vitro* growth of human pathogenic bacteria such as *S. aureus, P. aeruginosa, and E. coli*, in addition to exhibiting fungicidal and fungistatic activity on fungi genera *Mucor, Rhizopus, Aspergillus, Candida*, and *Penicillium*.

The use of ulmo honey in association with oral vitamin C as an alternative in healing treatment of *burn wounds* in guinea pigs (*Cavia porcellus*) improves regeneration in this type of wound and also reduces the possibility of infection, inflammation, and edema [49–51]. In addition, the clinical effect of topical treatment with ulmo honey associated with oral vitamin C in patients with venous ulcers was evaluated. This treatment method presented significant results, healing wounds faster in 100% of patients with all types of venous ulcers. Furthermore, the honey presented nonadherent and debriding properties was straightforward to apply and remove, and was well received by users [52].

Regarding honey of *Q. saponaria*, the antibacterial and antifungal activities were analyzed. Extracts of unifloral honeys of quillay were tested for antibacterial activity on *P. aeruginosa, E. coli, Staphylococcus typhi, S. aureus, Streptococcus pneumoniae* type β, and *Vibrio cholerae*, and antifungal activity against *Candida albicans*. The best *in vitro* activity of these extracts were on *S. aureus* and hemolytic *S. β*, both of which affect the skin [13]. The antibacterial effects exhibited could be related to an overall effect of the phenolic compounds present in the extract (caffeic, coumaric and salicylic acids, the flavanone naringenin and the flavonol kaempferol), which were detected by high-performance liquid chromatography.

Microbial resistance to honey has never been reported which makes it a very promising topical antimicrobial agent against the infection of antibiotic-resistant bacteria and in the treatment of chronic wound infections that do not respond to antibiotic therapy. The potency of honey, such as Chilean honey, against microorganisms suggests its potential to be used as an alternative therapeutic agent in certain medical conditions, particularly wound infection.

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