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Honey as a micro-bacterial agent: identification method of the compounds that inhibit pathogenic bacteria

Arias-De la Cruz, Heydi L.[1]; Zaldívar-Cruz, Juan M.[1*]; Ruiz-May, Eliel[2], Hernández-Nataren, Edith[1], Sol-Sánchez, Ángel[1], Nila-Méndez, Alejandro G.[3]

[1] Colegio de Postgraduados - Campus Tabasco, Periférico Carlos A. Molina, Tabasco, México, C. P. 86500.
[2] Instituto de Ecología A.C., Colonia El Haya, Xalapa, Veracruz, México, C. P. 91073.
[3] Tecnológico Nacional de México/ITS Acayucan, Colonia Agrícola Michapa, Acayucan, Veracruz, México, C. P. 96100.
* Correspondence: zaldivar@colpos.mx

ABSTRACT

Objective: To provide an overview of the scientific evidence that supports the use of *Apis mellifera* honey as an antibacterial agent.

Design/Methodology/Approach: An exhaustive review of scientific literature was carried out. The collected information included the different honey types that, according to the reports, have antibacterial properties. In addition, the related compounds, the main chromatographic methods used for their identification, and the main pathogens that have been studied were analyzed.

Results: The antibacterial properties of honey (especially monofloral honeys) have been widely studied worldwide, focusing on their capacity to inhibit pathogenic bacteria. The different methods used to study honey include agar diffusion, disk diffusion, and broth and agar dilution. These properties have been attributed to honey, as a result of its high sugar content, low moisture content, and acidic pH, as well as the diversity of the chemical compounds —mainly hydrogen peroxide, methylglyoxal (MGO), phenolic acids, flavonoids, peptides, glycopeptides, and different proteins— that were identified by a chromatographic analysis.

Study Limitations/Implications: Currently, the honey of bees (*Apis mellifera*) has great potential as an alternative to combat the antibiotic resistance of certain pathogens.

Findings/Conclusions: Honey can inhibit both gram-positive and -negative bacteria, such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomona aeruginosa*, and *Enterobacter*. These characteristics are the result of the diverse chemical compounds of honey. In addition, these compounds widely change depending on the vegetation that surrounds the hives; therefore, honey from different geographical origins has unique characteristics, in terms of its composition and antibacterial activity.

Keywords: *Apis mellifera*, honey, antibacterial property.

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INTRODUCTION

Honey is a sweet substance produced by bees, from the flower nectar or exudations of live parts of plants, that is transformed and stored in their honeycomb (Martínez-González and Pérez-López, 2013). This natural product consists of approximately 38% fructose, 31% glucose, 10% of different types of sugar, 18% water, and 3% of other compounds —such as

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proteins, lipids, amino acids, phenolic compounds, minerals, vitamins, and carotenoids (Hegazi *et al.*, 2014; Bueno-Costa *et al.*, 2016; Visweswara *et al.*, 2016; Deng *et al.*, 2018; Cheung *et al.*, 2019; Leyva-Jiménez *et al.*, 2019).

Honey is very important worldwide and it has been used to treat skin wounds, burns, sores, eye infections, sore throat, and other issues. The properties of honey are the consequence of its high thickness that protects against infections, its enzymatic production of hydrogen peroxide (H_2O_2), its high osmolarity, and its low pH (3.2-4.5). As a whole, these characteristics inhibit the growth of pathogenic bacteria (Mandal and Mandal, 2011). In addition, honey is consumed as a result of its high nutritional value and its antioxidant, bacteriostatic, and anti-inflammatory properties (Alvarez-Suarez *et al.*, 2014); these biological compounds are passed from the nectar to the honey and can be found in high levels (Güneş *et al.*, 2017).

The inappropriate use of antibiotics to treat bacterial infections has produced different degrees of bacterial resistance and, consequently, the use of these products has been limited. *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella enterica* are some of the hospital pathogens that cause difficult-to-treat infections; they have developed an increasing resistance to antibiotics, causing a worldwide concern (Da Silva and Mendonça, 2012; Fyfe *et al.*, 2017).

Consequently, finding new alternatives to improve human health is fundamental. Some of these alternatives include the combination of antibacterial formulations aimed to produce more efficient antibiotics or the search for natural alternatives, such as apitherapy. This last alternative offers honey-based treatments and other honeycomb products that can be used to combat several bacterial infections (Estrada *et al.*, 2005; Mandal and Mandal, 2011; Boussaid *et al.*, 2018). Jenkins and Cooper (2012) have reported that the oxacillin and Manuka honey synergy can be used to treat S. aureus, which is resistant to oxacillin. In addition, the Manuka honey and rifampicin (antibiotic) combination can be used to inhibit *S. aureus*, which is resistant to methicillin (MRSA). The antibiotic resistance reversion is a consequence of the reduction of the mecR1 (MRSA resistance gene) caused by honey (Müller *et al.*, 2013).

Consequently, the objective of this review was to gather scientific evidence about the properties of honey produced by bees (*Apis mellifera*) as an antibacterial agent and to identify the main methods used to establish the secondary metabolites involved in the antibacterial potential.

MATERIALS AND METHODS

A bibliographic review was carried out using the PubMed, Google Scholar, Scopus, and Science Direct databases, in order to gather information about the main methodologies used to evaluate the antibacterial properties of honeys from different geographical origins. In addition, the main secondary metabolites related with the antibacterial activity of honey and the chromatographic methods used to identify them, during the last twenty-five years, were integrated into the review. The search criteria were limited to English, Portuguese, and Spanish and were based on the scientific publications about antibacterial activity of *Apis mellifera* honey, antibacterial metabolites, and antibacterial properties identification

methods. The Mendeley Reference Manager was used to choose and download only those open access publications that included key information.

RESULTS AND DISCUSSION

The initial search identified 250 publications in the different abovementioned databases. Once all the publications were analyzed, 140 were excluded, because their content was not related to the objective of this study. Most of the chosen publications were experimental qualitative studies, which identified both international and domestic honey compounds and the equipment used for this purpose.

Methods and techniques used in the identification of antibacterial compounds

As a result of its many compounds, honey is a complex matrix; however, these compounds can be analyzed using chromatographic techniques. Pyrzynska and Biesayga (2009) tested methods and techniques to obtain higher extraction yields for the different chemical structures and functional groups (aldehydes, ketones, carboxylic acid, esters, alcohols, and flavonoids).

The chromatographic analysis requires a representative sample, which must be subjected to a pre-treatment (extraction) to remove any compound that could interfere in the analysis, such as sugars and polar substances. Highly polar compounds (flavonoids and phenolic acid) should be subjected to a derivatization (chemical changes in the analyte or sample) (Ciulu *et al.*, 2016).

Analytical tools, such as the UV-Vis spectrophotometer, the spectrofluorometer, and ready-to-use H_2O_2 assay kits has been used to measure the H_2O_2 level of honey (Sowa *et al.*, 2017).

Chen *et al.* (2012) evaluated the H_2O_2 content of processed (heat treatment) and unprocessed Australian honeys, measuring the absorbency at 560 nm, and found a 0-1,017 μM concentration. The correlation of the antibacterial activity was higher in the unprocessed samples, because the compound is sensitive to the heat treatment. Likewise, Sowa *et al.* (2017) determined the H_2O_2 content of monofloral clover honeys (Poland), using an absorbency of 540 nm. These samples recorded activity against gram-positive bacteria, with 12.5-25% concentrations.

Poli *et al.* (2018) evaluated the H_2O_2 concentrations of five honey samples, with and without catalase (an enzyme that catalyzes the decomposition of H_2O_2 into oxygen and water), and they recorded that honey without catalase obtained 7-8% minimum inhibitory concentration (MIC) values, proving its high antibacterial capacity. Meanwhile, when these authors added catalase to the samples, the values increased by 25%. These results were corroborated by Sindi *et al.* (2019), who recorded a 29.4% increase with honey treated with catalase.

The H_2O_2 assay kits are very reliable, given their sensibility in presence of peroxidase. The reagent of the kit reacts with H_2O_2, creating resorufin (a fluorescent red oxidation product). This reaction has been used to detect it in <100 μL volumes. Compared with other, cheaper, and easier-to-use colorimeter methods, the main disadvantage of this product is its high cost (Chen *et al.*, 2012; Sowa *et al.*, 2017).

A high-performance liquid chromatography (HPLC) was used to analyze methylglyoxal (MGO) as the quinoxaline content that remained after the o-phenylenediamine derivatization. Atrott and Henle (2009) recorded values that fluctuated between 189 and 835 mg kg^{-1}, showing a good methylglyoxal-antibacterial activity correlation (12.4 and 30.9%, respectively). For their part, Sultanbawa *et al.* (2015) used an ortho-phenylenediamine derivatization and recorded a lineal correlation between the MGO (279-1,755 mg kg^{-1}) content and the *E. coli* and *S. aureus* bacterial inhibition (8-55 mg kg^{-1} to inactivate the bacteria).

Cokcetin *et al.* (2016) used an O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine derivatization and obtained 1,100 mg kg^{-1}, recording a strong correlation between the concentration of the bacterial activity without peroxide and MGO. These results were corroborated by Rückriemen *et al.* (2017).

Other studies have used different methods to identify the phenolic compounds, including a HPLC with a PhotoDiode Array Detector (PDA) and a UV-Vis spectrophotometer. Additionally, they compare the retention time of the analytes with reference standards. Table 1 shows the analytical methods used to identify honey phenolic compounds in different countries. Escriche *et al.* (2014) used a HPLC-PDA to determine the flavonoids and phenolic acids of citrus, rosemary, and honeydew honeys and they identified the following indicators: hesperetin (citrus honey), pinocembrin (rosemary honey), and myricetin and p-coumaric acid (honeydew honey). Güneş *et al.* (2017) found caffeic, protocatechuic, and p-hydroxybenzoic acids in honeys from Turkey; while the most abundant compounds of these honeys were pinocembrin, chrysin, and galangina.

Apigenin, 4-hydroxybenzoic acid, isorhamnetin, luteolin, and pinocembrin were found in all honey samples from different regions of Algeria (Ouchemoukh *et al.*, 2017). Meanwhile, gallic acid (phenolic acid) and chrysin (flavonoids) were the major compounds found in commercial honeys from different countries (Cheung *et al.*, 2019). For their part, Elrasheid *et al.* (2017) detected vanillic, chlorogenic, syringic acid, and catechin in honey samples from Sudan. These bioactive compounds produce antibacterial, antioxidant, and anti-inflammatory effects (Leyva-Jiménez *et al.*, 2019; Goslinski *et al.*, 2020; Velásquez *et al.*, 2020).

Antibacterial properties of honey

The antibacterial properties of honey are the result of its high sugar content and its capacity to generate H_2O_2, produced by the glucose oxidase enzyme synthesized by bees. This compound destroys the essential components of the cells; however, the inhibitory capacity decreases as temperature rises. This phenomenon shows that the antibacterial potential of honey depends on its composition, the nectar source from which bees feed, the harvesting conditions, the pasteurization process, storage time, and temperature of the storing facilities (Fernandes *et al.*, 2020; Goslinski *et al.*, 2020; Velásquez *et al.*, 2020).

The efficiency of H_2O_2 as an antibacterial agent has been proved; nevertheless, in some honeys, it is related to low pH (3.2-4.5), cytokine release, and several molecules with immunomodulatory and anti-inflammatory properties (Almasaudi *et al.*, 2017; Leyva-Jiménez *et al.*, 2019; Fernandes *et al.*, 2020). For example, Melaleuca honey has an acid

Table 1. Main phenolic compounds of honeys from different geographical origins, detected by chromatography.

Country	Secondary metabolites	Honey	Method chromatographic	Reference
New Zealand and y Australia	Myricetin, crysin and chlorogenic, gallic, caffeic, ferulic, *p*-coumaric, rosmarinic, ellagic, 3,4-dihydroxybenzoic and abscisic acids.	Manuka *Leptospermum scoparium*	HPLC-DAD, HPTLC	Stanek y Jasicka-Misiak, 2018
Malaysia	Caffeic, chlorogenic, ferulic, *p*-coumaric, sinapic, vanillic and syringic acids.	*Melaleuca alternifolia*	RP-UHPLC-ESI-MS	Goslinski *et al.*, 2021
Poland	Chlorogenic and ferulic acid.	*Fagopyrum esculentum*, *Calluna vulgaris* and Tilia	RP-UHPLC-ESI-MS	Goslinski *et al.*, 2021
Poland	Myricetin, quercetin, naringenin, chrysin and acids: cinnamic, caffeic, abscisic and ferulic.	*Robinia pseudoacacia* L.	HPTLC	Stanek *et al.*, 2019
Bangladesh	Catechin, naringin, myricetin, naringenin, hesperetin, kaempferol, apigenin and acids: gallic, chlorogenic, caffeic, coniferous, benzoic and transcinnamic.	*Brassica nigra*, *Nigella sativa*, *Nelumbo nucifera*	HPLC-UV	Moniruzzaman *et al.*, 2014
Chile	Hymenoptaecin, rutin, naringerin, esculetin, scopoletin, and syringic, *p*-coumaric, and vanillic acids.	*Cryptocarya alba*, *Quillaja saponaria*	HPLC-MS-MS	Montenegro *et al.*, 2008; Velásquez *et al.*, 2020
China	Quercetin, apigenin, kaempferol, isorhamnetin, chrysin, galangin and protocatechuic, chlorogenic, caffeic, syringic, *p*-hydroxybenzoic, *p*-coumaric, ferulic, isoferulic and benzoic acids.	*Fagopyrum esculentum*	RP-HPLC-UV	Deng *et al.*, 2018
Spain	Hesperetin, kaempferol, chrysin, pinocembrin, myricetin, naringenin, quercetin, galangin and caffeic, chlorogenic and *p*-coumaric acids.	*Quercus ilex*, *Quercus robur*	HPLC-PDA,	Escriche *et al.*, 2014
Portugal	Chrysin, galangin, hesperidin, pinobanksin, luteolin, pinocembrin, kaempferol, apigenin and gallic, caffeic, coumaric and ellagic acids.	*Eucalyptus* spp., *Castanea sativa*, *Rubus* spp., *Erica* spp.	HPLC-DAD Infinity	Silva *et al.*, 2020
Saudi Arabia	Apigenin, chrysin, galangin, luteolin, myricetin, naringin and 4-hydroxybenzoic, syringic and gallic acids.	*Lavandula dentata*, *Hypoestes forskaolii*, *Ziziphus spina-christi*	HPLC-PDA	Badjah *et al.*, 2016
Australia	Apigenin, hesperetin, naringenin neoponcirin, narirutin, biochanin and rosmarinic, chlorogenic and caffeic acids.	*Eucalyptus marginata* and *Corymbia calophylla*	HPLC-PDA, HPLC-MS/MS	Tang *et al.*, 2016; Mani *et al.*, 2021
Türkiye	Apigenin, pinocembrin, chrysin, galangin, genkwanin and gallic, phydroxybenzoic, caffeic, syringic, salicylic, *p*-coumaric and transferulic acids.	*Castanea sativa*	HPLC-DAD	Güneş *et al.*, 2017
Soudan	Catechin and chlorogenic, syringic, caffeic, *p*-hydroxybenzoic, vanillic, *p*-coumaric, ferulic and cinnamic acids.	*Acacia nilotica*, *A. seyal*, *Z. spina-christi*, *Amaranthus graecizan*, *Eucalyptus* spp.	FTIR-ATR-PLSR, HPLC-DAD	Elrasheid *et al.*, 2017
Algeria	Gallic acid, and caffeic acid, chrysin, luteolin, pinocembrin, pinobanksin, myricetin, genistein and daidzein.	*Eucalyptus*, *Ziziphus*, *Euphorbia*, *Citrus* or *Hedysarum*	UPLC-PDA-MS/MS	Ouchemoukh *et al.*, 2017
International commercial honeys	Vanillin, syringaldehyde, protocatechualdehyde and gallic, protocatechual, 2,3,4-trihydroxybenzoic, *p*-hydroxybenzoic, gentic, chlorogenic, vanillic, caffeic, syringic, *p*-coumaric, ferulic, synapic, salicylic acids.	*Acacia* spp., *Tilia* spp, *Leptospermum scoparium*, *Eriobotrya japonica*	RP-HPLC-DAD Infinity	Cheung *et al.*, 2019

HPLC=High-Performance Liquid Chromatography; PDA=PhotoDiode Array Detector; UV=UV-Vis spectrophotometer; DAD=Diode Array Detector; FTIR=Fourier Transform Infrared Spectroscopy; HPTLC=High-Performance Thin Layer Chromatography; UPLC=Ultra Performance Liquid Chromatography; Ms/Ms (MS-MS)=Tadem Mass Spectrometry; UHPLC=Ultra-High-Performance Liquid Chromatography; RP=Reverse phase; ESI=Electrospray ionization; ATR=Attenuated total reflectance; PLSR=Partial least squares regression.

pH (3.7), perhaps due to the gluconic acid that causes anti-staphylococcal effects (Wen-Jie and Mei-Siew, 2015; Schencke *et al.*, 2016).

The methylglyoxal (MGO) and defensin-1 content of Manuka (*Leptospermum scoparium*) honey results in a high antibacterial activity against *Streptococcus pyogenes*, *Streptococcus mutans*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *S. aureus* (Irish *et al.*, 2011; Habib *et al.*, 2014; Johnston *et al.*, 2018; Matzen *et al.*, 2018). Consequently, Manuka honey is used as an alternative antibiotic to heal tissue and to reduce microbial infections (Johnston *et al.*, 2018).

Revamil®, a medical-grade honey, is produced under standardized conditions in greenhouses. Its potent and reproducible antibacterial activity is the result of its osmotic activity, acid pH, and slow and progressive H_2O_2 release. In addition, its MGO content creates favorable and optimal conditions to heal wounds (Kwakman and Zaat, 2012; Pharma GDD, 2022).

Leptospermum honey has high MGO concentrations. Atrott and Henle (2009) and Fernandes *et al.* (2020) have reported up to 800 mg kg^{-1} MGO content, which is 100 higher than the concentration found in common honey. MGO concentrations increase as the honey matures. This phenomenon is possibly the consequence of the non-enzymatic conversion of trioses that takes place during long-term storage (up to 120 days, at 37 °C). Meanwhile, its antibacterial effect comes from its capacity to inactivate proteins through cross-linking (Adams *et al.*, 2009).

The physiological wound healing processes can be divided into three phases: inflammatory, proliferative, and remodeling. During the inflammatory phase, honey stimulates the monocytes to release inflammatory cytokines. In the proliferative phase, angiogenesis and fibroplasia take place, along with initial re-epithelialization and contraction of the wound. Finally, during the remodeling phase —when collagen is remodeled and realigned along the tension lines—, apoptosis removes the cells that are no longer needed (Schencke *et al.*, 2016).

Polyphenols are major compounds that have been strongly linked to the antibacterial properties of monofloral honeys. In addition, they can be used to determine the floral and geographic origin of honeys (Iqbal *et al.*, 2015; Albaridi, 2019; Mama *et al.*, 2019; Cebrero *et al.*, 2020) and can be found mainly as flavonoids and phenolic acid and its derivatives (Figure 1) (Cauich *et al.*, 2015; Hossen *et al.*, 2017; Leyva-Jiménez *et al.*, 2019).

The identification and quantification of these compounds are fundamental to understand the bioactivity of honey (Habib *et al.*, 2014). Several authors have proved that darker honeys had high levels of phenolic compounds and, at the same time, high pharmacological potential (Brudzynski and Miotto, 2011; Sant'Ana *et al.*, 2014; Sousa *et al.*, 2016). Although individual phenolic compounds have antibacterial activity, their interaction with H_2O_2 should be taken into account, because it can promote this effect (Escuredo *et al.*, 2012; Poli *et al.*, 2018).

The high number of peptides help honey bees to protect themselves from diseases that impact the honeycomb. Honey has four antimicrobial peptide (AMP) families: defensins, apidaecins, abaecins, and hymenotaecin.

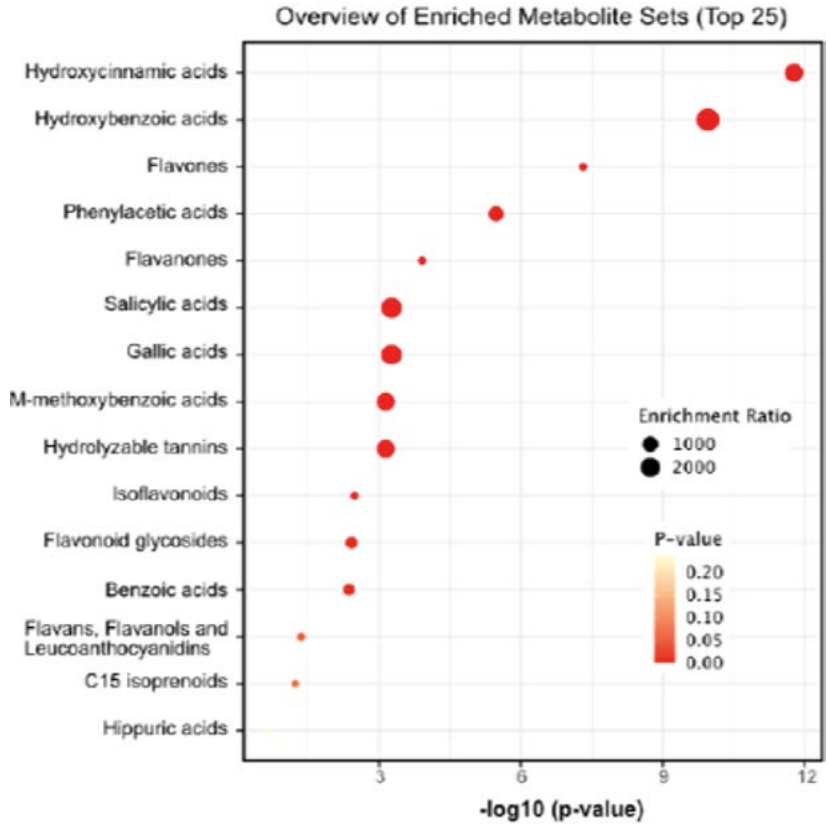

Figure 1. Analysis of the enrichment of secondary metabolites identified to date in honey.

Defensins act against gram-negative bacteria, gram-positive bacteria, and fungi. They are responsible for the destruction of the biofilm formed by the bacteria. Reports indicate that defensins amount to 0.04-5.17 μg g^{-1} honey (Kwakman and Zaat, 2012; Majtan *et al.*, 2012; Dosler and Karaaslan, 2014; Yi *et al.*, 2014; Valachová *et al.*, 2016). Nevertheless, some studies indicate that some gram-negative bacteria are resistant to defensin-1 (Čeřovský and Bém, 2014). Meanwhile, apidaecin is an antibacterial peptide with a high proline content. Once it enters the bacteria through the lipid bilayer, apidaecin stops the protein expression (Li *et al.*, 2012; Larsen *et al.*, 2019).

Abaecin can be found in western honey bees. It inhibits the growth of both gram-negative and gram-positive bacteria through the permeabilization of the bacteria membrane. Its expression and abundance quickly increase in response to a bacterial infection (Randolt *et al.*, 2008; Danihlík *et al.*, 2015; Larsen *et al.*, 2019). Finally, hymenoptaecin has an abundant glycine content that inhibits the growth of gram-positive and gram-negative bacteria (Erban *et al.*, 2019; Larsen *et al.*, 2019).

Antibacterial activity of honey

Different protocols have been used to evaluate the antibacterial activity of honey: agar diffusion test, disk diffusion test, broth and agar dilution method, and gradient methods (E-test and spiral plating technique) (Balouiri *et al.*, 2016; Osés *et al.*, 2016). The most used methods to determine the minimum inhibitory concentrations (MIC) required to

stop or eliminate bacterial growth are the disk diffusion, well diffusion, and broth and agar dilution methods. *S. aureus* is usually the bacterium of choice for tests and *in vitro* antibacterial activity, as a consequence of its resistance to high sugar content and acidity levels. In addition, this bacterium is sensitive to the action of H_2O_2 (Khalil *et al.*, 2014; Osés *et al.*, 2016).

Reproducibility tests are frequently difficult, since the work is carried out with living beings and several methods are used to determine the antibacterial activity of honey (Balouiri *et al.*, 2016; Osés *et al.*, 2016). However, reference material can provide a reliable base for lab work. In addition, reference strains can be used, because mutations can take place or, because depending on the method, the microorganisms can behave differently (Clavijo, 2002; Camaró-Sala *et al.*, 2015; Balouiri *et al.*, 2016; Roshan *et al.*, 2017; Albaridi, 2019; Mama *et al.*, 2019; Rosas *et al.*, 2019).

Chilean honeys, such as the Ulmo (*Eucryphia cordifolia*, Cav.) honey, are drawing attention due to their antibacterial potential. Velásquez *et al.* (2020) evaluated this type of honey using the agar diffusion method to determine their efficiency against *E. coli*, *P. aeruginosa*, and five *S. aureus*-MRSA strains and concluded that their antibacterial activity is mainly the consequence of their phenolic compounds (flavonoids, benzoic acid derivatives, and volatile compounds). Meanwhile, Cebrero *et al.* (2020) used the microdilution technique to evaluate the antibacterial activity of the Peumo (*Cryptocarya alba* (Molina) Looser) and Quillay (*Quillaja saponaria*, Molina) honeys against *S. aureus* and *P. aeruginosa*, pointing out that the antibacterial activity was the result of their hymenoptaecin content.

Roshan *et al.* (2017) analyzed the antibacterial activity in honey from western Australia. The results were compared with the results obtained by Activon, a medical-grade honey made from Manuka honey. The authors proved that six of the samples inhibited the growth of *S. aureus* and recorded larger inhibition areas than medical-grade honey. Meanwhile, Pasias *et al.* (2018) reported that Greek honeys had high H_2O_2 and hydroxymethylfurfural (HMF) levels. In addition, their acidity and osmolarity resulted in antibacterial activity against *S. aureus*.

For their part, Leyva-Jiménez *et al.* (2019) used the disk diffusion method to extract phenolic compounds from Iranian honeys, testing them against *E. coli*, *P. aeruginosa*, *S. aureus*, and *Enterococcus faecalis*. The authors concluded that individual and collective phenolic compounds do not have the same potential. These results showed a synergy between the different antibacterial compounds.

Hegazi *et al.* (2017) reported that 20 and 30% concentrations of honeys from Saudi Arabia inhibited *S. aureus*, *S. mutans*, *Klebsiella pneumoniae*, *E. coli*, and *P. aeruginosa* strains, as a result of their flower sources, osmotic properties, pH, H_2O_2, flavonoids, and antibacterial volatile substances (*e.g.*, organic acids). Mahendran and Kumarasamy (2015) used the disk diffusion method to evaluate the antibacterial potential of honey collected in Western Ghats, India, during summer and winter, against *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, and *P. mirabilis*. They concluded that the honey samples collected during winter had a higher bactericidal effect than those collected in summer, as a consequence of changes in seasonal flowering.

The eucalyptus honey from the Island of Mauritius is an exceptional case. It showed antibacterial activity against *E. coli*, *P. mirabilis*, *P. aeruginosa*, *Staphylococcus epidermidis* (ATCC 35984), and *S. epidermidis* (ATCC 14990). In addition, Aumeeruddy *et al.* (2019) reported that its phenolic compounds could eliminate cancer cells (MCF-7). Table 2 shows the microorganism inhibited by the different types of honey from several countries.

The processing and storage conditions can impact antibacterial properties of honeys from different geographical and botanical origin against various pathogens, because some of them lose their bactericidal effect under 40-60 °C temperatures (Chen *et al.*, 2012; Pimentel González *et al.*, 2017; Matzen *et al.*, 2018).

Overview of the antibacterial properties of Mexican honey

Honey plays a very important role worldwide not just as food, but also as a medical-grade product. The five main exporters of honey are China, Argentina, Mexico, Germany, and Brazil (Campos *et al.*, 2018). Beekeeping has a major socioeconomic and ecologic importance in Mexico, because this farming activity generates many jobs (Magaña *et al.*, 2016). Honey is very appreciated in several countries, as a result of its nutritional properties, its perfume, taste, and color; consequently, Mexican honey is currently competing with honey produced in China, Vietnam, Nicaragua, Argentina, Chile, Turkey, and Ukraine (Martínez-González and Pérez-López, 2013; Campos *et al.*, 2018).

In Mexico, researches have included different topics: botanical characterization (Córdova-Córdova *et al.*, 2013), physicochemical properties, volatile compound profiles (Viuda-Martos *et al.*, 2010; Rodriguez *et al.*, 2012), color (Figure 2) (Grajales-Conesa *et al.*, 2018), and antioxidant activity of harvested honey (Leyva-Daniel *et al.*, 2017; Mondragón, 2019). However, few studies have focused on the antibacterial properties of honey and the identification of the main compounds that provide Mexican honey with the said properties (Maddocks *et al.*, 2013).

Ramón-Sierra *et al.* (2020) compared the main phenolic compounds of honey produced by *Melipona beecheii* and *A. mellifera* from Merida, Yucatan. They found that the honey produced by *A. mellifera* had a higher phenolic compound and that 145 μg mL^{-1} (phenolic extracts) and 60 μg mL^{-1} (protein extracts) concentrations recorded antibacterial activities against *E. coli* and *S. aureus*. These results corroborated the findings of Chan-Rodríguez *et al.* (2012).

In addition, other studies have compared Mexican honey with honey from different countries. For example, some studies compare the honey of several honey bees (*Apis*) and the stingless bees from different countries such as Mexico (Jalisco, Chiapas, Quintana Roo, and Yucatan), Paraguay (Asuncion), Australia (Gatton and Brisbane), Philippines (Pili), Thailand (Chantaburi and Bangkok), Japan (Aichi), and Nepal. The evaluations were carried out to determine the effectiveness of those honeys against *Lactococcus lactis* ssp., *L. lactis cremoris* ssp. MAFF 40007, *Lactobacillus casei* JCM 1134, *E. faecalis* IFO 12964, *Enterococcus faecium* IFO 13712, *K. pneumoniae* ssp., and *Staphylococcus* ssp. The honeys from Yucatan and Chiapas (Mexico) recorded promising results in the inhibition of almost all the tested microorganisms, except for *K. pneumoniae* ssp. (Kimoto-Nira and Amano, 2008). Peláez-Acero *et al.* (2021) reported several new techniques to improve honey properties

Table 2. Minimum inhibitory concentration (MIC) of honeys from different geographical origins.

Country	floral source	Microorganisms that it inhibits	MIC (%)	Reference
Saudi Arabia	Dharm (*Lavandula dentata* L.), Majra (*Hypoestes forskaolii* (Vahl) R.Br.), Sider (*Ziziphus spina-christi* (L.) Willd)	*S. aureus*, *S. mutans*, *K. pneumoniae*, *E. coli* and *P. aeruginosa*.	20-30	Hegazi *et al.*, 2017.
Saudi Arabia	Yemeni Sidr and Mountain	*Gram-negative bacteria*	40-80	Alqurashi *et al.*, 2013
Algeria	Multifloral	*E. coli*	50-70	Bourabah *et al.*, 2020
Argentina	*Rapistrum rugosum* (L.) All., *Taraxacum officinale* F.H. Wigg.	*S. flexneri*, *Salmonella typhi*, *E. coli.*	100	Tejerina *et al.*, 2020
Australia	Jarrah (*Eucalyptus marginata* Donn ex Sm.) y Marri (*Corymbia calophylla* (Lindl.) KD Hill & LAS Johnson)	*Candida* spp. and *dermatophytic fungi*.	4-32	Irish *et al.*, 2011.
Chile	Ulmo (*Eucryphia cordifolia* Cav.)	*P. aeruginosa*, *S. aureus*, *E. coli* and *S. typhimurium*	9.4-20	Mullai y Menon, 2007; Sherlock *et al.*, 2010; Acevedo *et al.*, 2017; Velásquez *et al.*, 2020., Olate-Olave *et al.*, 2021
Denmark	Raspberry (*Rubus idaeus* L.), rapeseed (*Brassica napus* L.), Linden (*Tilia* L.) and Heather (*Calluna vulgaris* (L.) Hull)	*S. epidermidis* and *E. coli*.		Matzen *et al.*, 2018.
Ecuador	Multifloral	*S. aureus*	100	Montero-Recalde *et al.*, 2018
Egypt	Trefoil (*Trifolium repens* L.)	*S. typhimurium* and *E. coli* O157: H7	10-20	Mandal y Mandal, 2011.
Ethiopia	Multifloral	*S. aureus*	70-50	Mama *et al.*, 2019.
Ethiopia	Tazma mar (melipona), multiflorales	*S. aureus*, *P. aeruginosa* and *E. coli*.	9.38-11.25	Getaneh *et al.*, 2013.
Greece	Conifers, thyme (*Thymus* L.), citrus and multifloral	*S. typhimurium*	17-24	Voidarou *et al.*, 2011
India	Multifloral	*S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa* and *P. mirabilis*		Mahendran y Kumarasamy, 2015.
Mauritius Island	Eucalyptus (*Eucalipto globulus* Labill.)	*E. coli* ATCC 25922, *P. mirabilis* ATCC 12453, *P. aeruginosa* ATCC 27853, *S. epidermidis* ATCC35984, and *S. epidermidis* ATCC1499	100	Aumeeruddy *et al.*, 2019.
Malaysia	Tualang (*Koompassia excelsa* (Becc.) Taub.)	*E. coli*, *S. typhi* and *S. pyogenes*.	8.75-25	Mandal y Mandal, 2011.
Mexico	Acaxochitl (*Lobelia laxiflora* Kunth), multifloral	*B. subtilis*, *S. aureus, L. monocytogenes*, *E. coli*, *S. typhimurium* and *P. aeruginosa*.	55-100	Rodriguez *et al.*, 2012; Pimentel González *et al.*, 2017.
New Zeeland	Manuka (*Leptospermum scoparium* J.R. Forst. and G. Forst.)	*S. pyogenes*, *S. mutans*, *P. mirabilis*, *P. aeruginosa*, *E. cloacae* and *S. aureus*.		Irish *et al.*, 2011; Johnston *et al.*, 2018.
Pakistan	Multifloral	*E. coli*, *P. aeruginosa*, *S. aureus*, *E. faecalis*.	75-100	Mustafa *et al.*, 2022
Portugal	Multifloral, *Leptospermum scoparium*	*E. coli* CECT 434, *E. coli* EC3a	25	Oliveira *et al.*, 2017
United Kingdom	Heather (*Calluna vulgaris*)	*S. aureus*, *E. coli*, *E. faecalis* and *P. vulgaris*.	2.5-40	Vučić *et al.*, 2014.

Figure 2. Color range of honey samples from Tabasco, Mexico.

(crystallization). These authors used an ultrasound to study the effect of liquefaction in the phenolic compounds, flavonoids, and antibacterial activity of crystalized honey from three multifloral honeys from Hidalgo. Their antibacterial activities were tested against *S. typhimurium*, *B. subtilis*, *P. aeruginosa*, *L. monocytogenes*, *S. aureus* and *E coli*. Honeydew honey recorded a higher gallic acid content and some multifloral samples increased their quercetin content; meanwhile, all the samples inhibited the tested microorganisms. In particular, the antibacterial activity against *S. typhimurium* increased by 13%.

Overall, monofloral honeys have a higher inhibition range against pathogens than multifloral honeys (Oelschlaegel *et al.*, 2012; Rodriguez *et al.*, 2012; Escriche *et al.*, 2014; Pimentel-González *et al.*, 2017; Aumeeruddy *et al.*, 2019; Olate-Olave *et al.*, 2021).

Limitations

Currently, the use of honey for therapeutic effects has increased. It can be used for wound healing and to treat gastrointestinal diseases and eye infections. Nevertheless, evaluating the quality of the available scientific evidence is fundamental. On the one hand, information about the sample size is limited; on the other hand, the methods used to identify the properties of honey and the sample extraction before chromatography are not standardized. In addition, processing methods (pasteurized or unpasteurized) should be taken into account and the range of pathogenic bacteria evaluated should be expanded.

CONCLUSIONS

The antibacterial activity of honey has been used since ancient times to treat different health problems. Studying honey has never been an easy task: it is a complex system with inherent characteristics and many chemical compounds that change depending on the types of honey and its botanical and geographical origin. Overall, honeys produced from specific botanic sources have a high antibacterial activity, as a consequence of the medicinal properties of the flower source that are transferred to the honey through the nectar.

The efforts of the scientists to determine the underlying mechanisms of the bactericidal effect of honey have paid off. Scientists have proved that the said activity is related to the pH level, the osmotic effect caused by the high sugar content, the hydrogen peroxide content, the methylglyoxal content, the phenolic compounds, and the flavonoids that can work together or individually, depending on the type of honey (floral source, geographical

origin, etc.). All these characteristics help to stop the growth of certain pathogens and, consequently, honey has been used in the health sector to treat several diseases. Nevertheless, the antibacterial activity must be confirmed to prove the medical grade of a given honey. Subsequently, optimal production, handling, harvesting, and storage procedures should be established, in order to guarantee the safety of the product —*i.e.*, honey should be free of pathogens and toxic compounds. Finally, the effects of the infection and disease treatment should be proved through clinical trials.

The protocols used to study the phenolic compounds are different and are related to the detector that will be used with the HPLC. This selection is directly related to the analytes that will be identified and the budget established for the research. New technologies have led to promising results, reducing costs and analysis time; however, new technologies should be focused on the analysis of the metabolites of honey.

Consequently, further research about the different components of honey should be carried out to find new alternatives that reduce or stop antibiotic resistance. These studies should result in the use of honey as a substitute or its combination with classic antibiotics. Nevertheless, establishing standardized methodologies among the scientific community will help to determine the antibacterial activity of honey, facilitating its analysis and expanding the research of this property among honeys of different botanical origins.

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