

Honey as a Complementary Medicine

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ABSTRACT: The beneficial effects of honey on human health have long been recognized. Today, many of those positive effects have been studied to elucidate its mode of action. This review briefly summarizes the best studied features of honey, highlighting it as an appealing alternative medicine. In these reports, the health benefits of honey range from antioxidant, immunomodulatory, and anti-inflammatory activity to anticancer action, metabolic and cardiovascular benefits, prebiotic properties, human pathogen control, and antiviral activity. These studies also support that the honey's biological activity is mainly dependent on its floral or geographic origin. In addition, some promising synergies between honey and antibiotics have been found, as well as some antiviral properties that require further investigation. Altogether, these studies show that honey is effectively a nutraceutical foodstuff.

KEYWORDS: Honey, pathogens, biofilm, prebiotic, metabolism, cancer, heart

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Introduction

Honey is produced by bees from the genus *Apis*, which collect the nectar from plants or from secretions of aphids (plant-sucking insects belonging to the genus *Rhyncho*).¹ It is a sweet and flavourful natural product, supersaturated in sugars, with high nutritive value. Besides the sugars, other minor components are present in honey, such as minerals, polyphenols, vitamins, carotenoids, amino acids, proteins, enzymes (glucose oxidase and catalase [CAT]), organic acids, and volatile compounds. In addition to the described prebiotic effects associated with oligosaccharides,² most of the reported biological properties of honey (eg, antioxidant, antibacterial, antiviral, anti-inflammatory, antiulcerous, immunomodulating, vasodilative, hypotensive, antihypercholesterolemic, antibrowning, disinfectant, and antitumour), and many of its applications, may be attributed to those minor components.^{2–4} However, each honey's particular composition is highly dependent on the floral source, geographical region, and season, as well as the processing conducted after the harvest.^{3,5}

Chemical Composition

Honey is mainly constituted by sugars and in much lower quantities by amino acids, proteins, enzymes, organic acids, vitamins, minerals, volatile substances, and polyphenols.⁶

Fructose and glucose (monosaccharides) represent about 75% of the sugars found in honey. Disaccharides (sucrose, maltose, turanose, isomaltose, maltulose, trehalose, nigerose, and kojibiose) and trisaccharides (maltotriose and melezitose) among other oligosaccharides constitute the remaining 10% to 15% of the carbohydrates in honey.^{5,6}

Usually, fructose is honey's most predominant sugar, with acacia being one of the plants that yields the highest amount of

this monosaccharide.^{7,8} Nevertheless, there are exceptions, eg, honey from rape (*Brassica napus*) and dandelion (*Taraxacum officinale*) has higher concentrations of glucose than fructose.⁷ These findings suggest that carbohydrates may play a role in the identification of botanical and/or geographical origins of monofloral honeys, along with other parameters, such as water activity and electrical conductivity.⁹ Beyond the concentrations of glucose and fructose, their ratio may also contribute to classifying monofloral honeys, as well as the concentrations of some minor oligosaccharides and/or their combinations.^{7–9}

Proteins (0.5%) are present in honey mainly not only as enzymes (diastase or amylase, invertase or sucrase or α -glucosidase, CAT, and glucose oxidase) but also as individual amino acids (proline is the most important one, along with more than 20 amino acids).^{5,6}

Organic acids (0.57%) include gluconic (the most abundant), aspartic, butyric, citric, acetic, formic, fumaric, galacturonic, gluconic, glutamic, glutaric, butyric, glyoxylic, 2-hydroxybutyric, α -hydroxyglutaric, isocitric, α -ketoglutaric, lactic, malic, malonic, methylmalonic, 2-oxopentanoic, propionic, pyruvic, quinic, shikimic, succinic, tartaric, oxalic, levulinic, and formic acids, among others.⁵

Honey also contains small amounts of vitamins: thiamine (B₁), riboflavin (B₂), nicotinic acid (B₃), pantothenic acid (B₅), pyridoxine (B₆), biotin (B₈ or H), folic acid (B₉), and vitamin C.⁵

The mineral content in honey ranges from 0.04%, in light honeys, to 0.2%, in dark honeys. Potassium is the most abundant element. Other macroelements and microelements may also be present in honey: magnesium, calcium, iron, phosphorus, sodium, manganese, iodine, zinc, lithium, cobalt,



nickel, cadmium, copper, barium, chromium, selenium, arsenic, and silver.⁵

The volatile compounds include diverse chemical groups, such as monoterpenes, C₁₃-norisoprenoid, sesquiterpenes, benzene derivatives and, to a lower content of superior alcohols, esters, fatty acids, ketones, and aldehydes.⁵

Some volatile compounds can be used for determining floral sources and/or geographical origins of honeys. For example, methyl anthranilate and sinensal isomers were identified only in citrus honey samples,^{10,11} and *cis*-linalool oxide, nonanol, and decanal were identified in acacia honey.¹² The volatile profile has been studied particularly in chestnut, heather, eucalyptus, and citrus from diverse regions, and the results have demonstrated some variability, even in the same region. However, the botanical and geographical origins are more easily achieved when the volatile compounds of hundreds of honey samples are analysed, along with their physicochemical properties, and then analysed by clustering, principal components analysis, and stepwise discriminant analysis. According to Oroian et al,¹³ this approach allowed the classification of honeys with more than 80% accuracy.

Polyphenols in honey may include phenolic acids (vanillic, caffeic, syringic, *p*-coumaric, ferulic, ellagic, 3-hydroxybenzoic, chlorogenic, 4-hydroxybenzoic, rosmarinic, gallic, and benzoic acids, among others) and flavonoids (quercetin, kaempferol, myricetin, pinobanksin, pinocembrin, chrysin, galangin, hesperetin, and others).⁵

The chemical diversity of phenols is also highly dependent on the botanical and geographical origins of honey. Several studies have proposed chemical markers for botanical origin taking into account the presence and abundance of one or more specific phenols.¹⁴ Table 1 presents the phenolic compounds which have been proposed as possible markers for inferring the honey's botanical origin.

The certification of the floral origins for monofloral honeys based exclusively on phenols, including the flavonoids' profile, is not enough. From Table 1, it is possible to ascertain that in some cases, for diverse floral origin of honeys, the same phenolic compounds can be found. According to Yao et al²¹ and Stephens et al,²⁴ the differences lie on the percentages and/or proportions between such compounds, which hamper the floral origin authentication of honeys. In addition, although Ferreres and colleagues^{26,27} considered quercetin and kaempferol as biomarkers for sunflower and rosemary honeys,^{26,27} there were other studies that considered those not to be adequate because there are also relatively high levels of these 2 flavonoids in melon, pumpkin, rapeseed, and cherry blossom honeys.²⁸ Some more examples have been reported: methyl syringate and lumichrome²⁹ present in manuka honey, considered as biomarkers, are also present in cornflower, thistle, *Satureja subspicata* Vis., and sage honeys.^{30–33} Therefore, other chemical parameters have been used for helping with the identification of the botanical and/or geographical origins of

monofloral honeys: metal ions, carbohydrates, amino acids, or volatile compounds.^{9,34–37}

Beyond the identification of the honey's floral origin, some authors have also reported that phenols may also be useful for determining its geographic origin. Gambacorta et al³⁸ showed that the phenolic profile of sulla's (*Hedysarum* sp) honey, produced in 8 different areas of Southern Italy, was influenced by their geographical origins, particularly the variations in the concentrations of gallic, chlorogenic, caffeic, *p*-coumaric, and ferulic acids, which were dependent on each honey's production place.

This kind of work requires an adequate number of samples, and large data sets are oftentimes difficult to be properly analysed and correctly interpreted. For this reason, several researchers have recently followed a chemometric approach to maximize information from chemical systems. For example, Karabagias et al³⁹ differentiated Greek thyme honeys according to their geographical origin, based on physicochemical parameters and phenolic profile, using multivariate analysis of variance and linear discriminant analysis. Other good example is that of Zhao et al⁴⁰ that using principal components analysis and discriminant analysis selected common chromatography peak areas of phenolic acids, allowing them to correctly classify more than 85% of the honey samples from 18 different areas of China. After analysing several phenolic profiles and total phenolic contents, the composition of minerals, sugars, and antioxidant activity of 18 unifloral *Salvia officinalis* honey samples, by applying principal components analysis to the data, they could accurately certify those honeys.³⁷

Most of the biological properties are attributed to the phenol components and, to a lesser extent, to some volatile compounds. However, other biophysical factors may also be involved: the glucose oxidase system (forms hydrogen peroxide), high osmotic pressure, acidity, low redox potential, high carbon to nitrogen ratio, bee defensin-1, and viscosity.^{34,41}

Antioxidant Activity

In a recent review,⁴² it became evident that honey possesses antioxidant activity, both in vitro and in vivo, independently from its floral or geographical origin. The in vitro assays show that honey is able to scavenge free radicals (1,1-diphenyl-2-picrylhydrazyl, peroxy radicals [oxygen radical absorbance capacity], 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulphonic acid], nitric oxide [NO]), as well as reduce ferric cations, chelate metal ions, inhibit lipid peroxidation measured by thiobarbituric acid reactive substances, and inhibit β -carotene bleaching.^{42–46}

In addition, the in vivo studies reveal that honey is able to stimulate the antioxidant defence system in the tissues of mice and rats (namely, pancreas, serum, kidney, and liver), particularly enhancing the activities of cellular antioxidant enzymes, such as superoxide dismutase, CAT, glutathione peroxidase, and glutathione *S*-transferase, and increasing the levels of reduced glutathione.⁴²

Table 1. Potential biomarker compounds (particularly phenols) for pinpointing the botanical origins of unifloral honeys.

FLORAL ORIGIN	GEOGRAPHICAL ORIGIN	COMPOUNDS	TECHNIQUE	REFERENCE
<i>Erica</i> sp (heather)	Portugal (Coimbra)	Ellagic acid, myricetin 3'-methyl ether	HPLC-DAD	Ferrerres et al ¹⁵
<i>Erica</i> sp (heather)	Portugal (Serra da Lousã)	Ellagic, <i>p</i> -hydroxybenzoic, syringic and <i>o</i> -coumaric acids	HPLC-DAD	Andrade et al ¹⁶
<i>Erica</i> sp (heather)	Not reported	Ellagic acid	CZE-DAD	Andrade et al ¹⁷
<i>Erica</i> sp (heather)	Germany, Denmark, Italy, Spain, France, The Netherlands, United Kingdom, Portugal	DL-Phenyllactic acid, phenylacetic and benzoic acids, benzoic acids	HPLC-UV	Dimitrova et al ¹⁸
<i>Lavandula stoechas</i> L. (lavender)	Portugal (Serra da Lousã)	Gallic acid	HPLC-DAD	Andrade et al ¹⁶
<i>Lavandula stoechas</i> L. (lavender)	Not reported	Naringenin	CZE-DAD	Andrade et al ¹⁷
<i>Lavandula</i> sp (lavender)	Germany, Denmark, Italy, Spain, France, The Netherlands, United Kingdom, Portugal	Gallic acid, caffeic acid	HPLC-UV	Dimitrova et al ¹⁸
<i>Thymus capitatus</i> Hoffgg. and LK. (thyme)	Not reported	Rosmarinic acid	CZE-DAD	Andrade et al ¹⁷
<i>Eucalyptus</i> sp	Italy, Portugal, Spain (all commercial)	Myricetin, tricetin, luteolin	HPLC-DAD	Martos et al ¹⁹
<i>Eucalyptus</i> sp	Spain and Italy	Myricetin, tricetin, and luteolin	LC-MS/MS	Truchado et al ²⁰
<i>Eucalyptus intermedia</i> (bloodwood)	Australia	Myricetin, tricetin, luteolin, quercetin, 2 unknown flavonoids	HPLC-DAD	Yao et al ²¹
<i>Eucalyptus ochrophloia</i> (yapunyah) and <i>Eucalyptus moluccana</i> (gum top)	Australia	Tricetin, luteolin, quercetin	HPLC-DAD	Yao et al ²¹
<i>Eucalyptus crebra</i> (narrow-leaved ironbark)	Australia	Luteolin, tricetin, quercetin, unknown	HPLC-DAD	Yao et al ²¹
<i>Eucalyptus nubila</i> (blue top ironbark)	Australia	Luteolin, tricetin, quercetin, myricetin, unknown	HPLC_DAD	Yao et al ²¹
<i>Eucalyptus globoidea</i> (stringybark) and <i>Eucalyptus largiflorens</i> (black box)	Australia	Tricetin, luteolin, isorhamnetin	HPLC-DAD	Yao et al ²¹
<i>Arbutus unedo</i> (strawberry tree)	Italy (Sardinia)	Homogentisic acid	HPLC-DAD	Cabras et al ²²
<i>Arbutus unedo</i> (strawberry tree)	Italy (Sardinia)	Homogentisic acid, (±)-2- <i>cis</i> ,4- <i>trans</i> -abscisic acid (<i>c,t</i> -ABA), (±)-2- <i>trans</i> ,4- <i>trans</i> -abscisic acid (<i>t,t</i> -ABA), unedone	HPLC-DAD, LC-MS/MS	Tuberoso et al ²³
<i>Kunzea ericoides</i> (kanuka)	North Island of New Zealand	4-Methoxyphenyllactic acid, phenyllactic acid, methyl syringate, methoxylated benzoic acid, 2-methoxybenzoic acid, structural isomer of syringic acid, trimethoxybenzoic acid	LC-MS/MS; GC-NPD	Stephens et al ²⁴
<i>Castanea sativa</i> Miller (chestnut)	Germany, Denmark, Italy, Spain, France, The Netherlands, United Kingdom, and Portugal	4-Hydroxybenzoic, DL- <i>p</i> -hydroxyphenyllactic, ferulic, phenylacetic acids	HPLC-UV	Dimitrova et al ¹⁸
<i>Helianthus annuus</i> L. (sunflower)	Germany, Denmark, Italy, Spain, France, The Netherlands, United Kingdom, and Portugal	<i>p</i> -Coumaric, ferulic, caffeic acids	HPLC-UV	Dimitrova et al ¹⁸
<i>Tilia</i> sp (Lime)	Germany, Denmark, Italy, Spain, France, The Netherlands, United Kingdom, and Portugal	3-Hydroxybenzoic acid	HPLC-UV	Dimitrova et al ¹⁸
<i>Asphodelus microcarpus</i> Salzm. et Viv. (asphodel)	Italy (Sardinia)	Methyl syringate	LC-MS/MS; HPLC-DAD	Tuberoso et al ²⁵

Abbreviations: CZE-DAD, capillary zone electrophoresis diode array detector; GC, gas chromatography; GC-NPD, gas chromatography with a nitrogen-phosphorus detector; HPLC-DAD, high-performance liquid chromatography-diode array detector; LC-MS/MS, liquid chromatography tandem mass spectrometry.

A strong correlation between the antioxidant activity of honeys and total phenolics (benzoic acids and their esters, cinnamic acids and their esters, and flavonoids and flavonoid aglycones) has been detected.⁴² However, the antioxidant activity must be considered as the result of a combined effect of several compounds present in honeys, which depend on floral and geographical origins, among other factors.^{45–48}

In the presence of oxidizing components (eg, hydrogen peroxide or transition metal ions), polyphenols may covalently bind to proteins after polyphenol auto-oxidation in the presence of O₂. This bond causes a decrease in either antioxidant activity or pro-oxidant activity.⁴⁹ According to Rice-Evans et al,⁵⁰ this loss of activity can be attributed to the chemical structure of phenols, particularly flavonoids, which present a catechol group on the B-ring, the 2,3-double bond in conjugation with a acarbonyl group at position 4 in the C-ring, and, finally, the presence of hydroxyl groups at positions 3 and 5 of the A-ring (eg, quercetin, myricetin, and quercetagenin). These chemical features also explain the antioxidant activity of flavonoids.⁵¹ In essence, the antioxidant or pro-oxidant activities may be attributed to the same flavonoids. The presence of hydrogen peroxide, metal ions, or oxygen, which can react with flavonoids, may direct them towards a pro-oxidant role.^{49,50}

The type of storage of the honey and/or its heat treatment increases the interaction between proteins and polyphenols, hence allowing the production of 2 types of complexes with distinct antioxidant power: the complexes with higher antioxidant activity are enriched for proteins with higher molecular weight, whereas the other complexes, enriched in polyphenols with lower molecular size, have lower antioxidant activity.⁵²

When honey is stored over long periods of time, or when submitted to heating, high-molecular-weight sugar-derived protein adducts are formed, with appear brown, known as melanoidins. In honey, polyphenols can also be included into melanoidins.^{53–55} The incorporation of polyphenols into these brown pigments may result in a decrease in its antioxidant activity^{54,56}; although in some cases such inclusions were responsible for either an increase or a decrease in the antioxidant activity,⁵⁵ depending on the colour of the honey. When heated, light and medium honeys gained antioxidant activity, in contrast to the dark ones, which show reduced antioxidant activity in response to heating.⁵⁵

Antioxidant properties have also been attributed to gluconic, malic, and citric acids, which act by chelating metal ions and increasing the effect of the antioxidant activity of flavonoids. Moreover, glucose oxidase and CAT are also reported to display antioxidant activity,³ as well as other compounds commonly found in honey, such as some of its mineral components,^{45,57,58} proteins,⁵⁷ and even some amino acids, including proline^{57,59}; although some studies have shown no direct correlation between antioxidant activity and the levels of proline.^{45,46}

The antioxidant activity of monofloral honeys from diverse geographical origins has hardly been studied, particularly

regarding its capacity for scavenging free radicals (in vitro assays), which have recently been compiled.¹⁴

Due to its antioxidant activity, honey has been suggested as an alternative to the sulphites used to inhibit the action of polyphenol oxidases, which in the presence of oxygen give rise to undesirable brown pigments in fresh-cut fruits and vegetables in the fruit juice processing industry.^{3,60,61} The main advantage of using honey instead of sulphites is the prevention of potential asthma attacks or anaphylactic reactions induced by sulphites in susceptible individuals.³

Oxidative stress and inflammation are closely interrelated, and therefore, the antioxidant effect of honey may also contribute to its anti-inflammatory activity.⁴²

Immunomodulatory Activity of Honey in Wound Healing

The process of wound healing is generally divided into 4 overlapping stages: haemostasis (seconds to minutes), inflammation (3–5 days), proliferation (4–14 days), and remodelling (8 days to 1 year). After haemostasis, and during inflammation, debris and bacteria are removed, followed by the proliferative stage, which is characterized by blood vessel invasion and regeneration of the connective tissue and epithelium, and finally, wound contraction. In the remodelling stage, collagen is rearranged, and excess tissue is removed by apoptosis.⁶² After the haemostasis stage, the tissue regeneration during wound healing starts with an immunostimulatory or inflammatory action as a defensive response to trauma and/or any pathogenic agents.⁴ However, in the presence of an unregulated inflammation, and particularly if this stage persists, a full recovery does not occur, giving rise to a chronic wound.⁴

Honey has been reported to display a dual role: (1) immunostimulatory or inflammatory action (stimulates the production of immunological mediators, such as tumour necrosis factor α [TNF- α], interleukin [IL]-1 β , and IL-6, and induces the upregulation of prostaglandin E₂ and cyclooxygenase-2 [COX-2]),^{63–69} and (2) anti-inflammatory action (suppresses the production of certain molecules, such as matrix metalloproteinases [MMPs], reactive oxygen intermediates, and reactive oxygen species [ROS], inhibits the expression of TNF- α and COX-2, attenuates the activation of nuclear factor- κ B [NF- κ B], or reduces NO production).^{4,70} These properties have all been correlated with the floral origin of each honey.^{70,71}

Over time, honey has frequently been used in the treatment of wounds and/or skin disorders (eg, eczema, dermatitis, burns, ulcers, and Fournier gangrene), not only for its antimicrobial abilities but also due to its other reported properties, such as the immunostimulatory or inflammatory activities, promotion of autolytic debridement, stimulation of wounded tissue growth to hasten healing and promote the healing process in dormant wounds, as well as its described anti-inflammatory features.^{62,63,70–72}

Table 2 presents some references describing the immunostimulatory properties of honeys of different botanical and/or geographical origins. Despite these reports, in some cases, an anti-inflammatory activity was also observed, inhibiting the release of some factors (cytokines, matrix metalloproteinase 9 [MMP-9], or ROS), either from immune or cutaneous cells, depending on wound conditions (acute or chronic inflammation), concentration, and type of honey.^{73–76} It has been hypothesized that at low concentrations of inflammatory/stimulatory mediators, honey exerts a pro-inflammatory role via the stimulation of production of inflammatory cytokines and MMP-9. Nevertheless, when the inflammation is already in progress, and in the presence of an infection, honey might act as an anti-inflammatory compound, inhibiting the production of inflammatory cytokines and MMP-9.⁷⁶

The assignment of immunostimulatory abilities to the respective compounds present in honey has been controversial. Although Timm et al attributed the induction of cytokines to the lipopolysaccharide (LPS) from contaminating bacteria present in honey,⁷⁹ other authors consider that LPS has no role in honey's immunostimulatory activity.^{66,67,69} Instead, other constituents are responsible for such feature, namely, type II arabinogalactans,⁶⁸ methylglyoxal (MGO),⁷⁴ and the major royal jelly protein 1 (MRJP1).⁶⁷ Nevertheless, it is undisputed that the immunostimulatory activity of honey in wound healing is influenced by the pH and the release of hydrogen peroxide.^{72,80}

This anti-inflammatory activity has been studied (1) in rabbits injected with LPS,⁸¹ (2) in the AGS cell line derived from a human gastric adenocarcinoma with *Helicobacter pylori*,⁸² (3) in rats with experimentally induced inflammatory bowel disease,⁸³ (4) in alkali injury on rabbits' eyes,⁸⁴ and (5) in rats with ethanol-induced gastric ulcers,⁸⁵ and several other examples previously compiled.⁴

More recently, considering only 2016, several authors reported in vitro and in vivo assays showing the anti-inflammatory activity of honey. For example, lavender honey shows better in vitro antihyaluronidase activity than citrus or strawberry tree honey, whereas the latter 2 perform better in inhibiting the lipoperoxidase activity.⁸⁶ The ability for inhibiting hyaluronidase in vitro has also been previously reported,⁸⁷ where oak, chestnut, and heather honeys displayed the best activity. The honey-induced inhibition of this enzyme was enhanced in combination with propolis.⁸⁸ In vivo testing with laboratory animals has shown a gastroprotective effect of manuka honey against ethanol-induced gastric ulcers in rats by decreasing the concentration of plasma inflammatory cytokines such as TNF- α , IL-1 β , and IL-6.⁸⁵ Moreover, multifloral honey was shown to downregulate the oxidative and inflammatory markers (decreased levels of tissue TNF- α and inducible nitric oxide synthase) in rats with colitis induced through dextran sodium sulphate administration.⁸⁹ However, other studies revealed no effect of honey when given to rats with colitis induced by acetic acid.⁹⁰

Several factors have been reported to contribute to the healing of wounds promoted by the usage of honey: hydrogen peroxide, glucose oxidase, gluconic acid, MGO, and polyphenols, along with its physical properties such as hygroscopicity, hypertonicity, and lower pH.^{4,76,91,92} However, the anti-inflammatory activity of honey on wounds starts with microbial elimination. Because they are the main source of inflammation, honey can act as a direct anti-inflammatory compound through several alternative mechanisms.

Owing to its antimicrobial, antioxidant, and anti-inflammatory activities, honey has long been used in wound healing (burns, ulcers, and others).⁹³ Several studies have reported the great potential of this bee product for wound healing: either applied alone^{94,95} on rat wounds or in association with other natural products (*Aloe vera* and milk or ascorbic acid), tested on burns in guinea pig and second-degree burns in rats,^{96,97} as chitosan nanofiber wound dressing enriched with *Allium sativum* and *Cleome droserifolia* applied on mice's wounds.⁹⁸

The benefits of honey in healing different types of human wounds have also been reported and reviewed in a recent publication,⁹³ although other contradictory reports show no additional benefit in using honey for some wound types.⁹⁹

The effectiveness of honey on wound healing has undergone some criticisms, particularly for the following reasons: poor study design, heterogeneous group of patients included in the studies, results not directly comparable between studies due to the usage of different methods and/or non-validated measurement methods, lack of appropriate blinding in the experiments, not enough study replication efforts, and usage of not so relevant comparisons which would help in making a clinical decision.^{93,100,101} Altogether, these factors hamper a definitive conclusion regarding the effectiveness and benefits of honey for healing wounds, independent of its floral or geographic origin.¹⁰¹

Anticancer Activity

The potential effects of honey on both the prevention of cancer and of tumour development and progression have been studied. Most of these studies have been performed in vitro. When tested on several types of human cancer cell lines (breast, prostate, endometrial, cervical, lung, skin, kidney, bladder, liver, oral squamous cell carcinoma, and osteosarcoma), honeys from diverse floral sources (*Abies cephalonica*, *Acacia*, *Citrus*, *Erica arborea*, forest, multifloral, *Pinus* sp, rosemary, thyme, and tualang tree) have demonstrated potential anticancer activity.^{102–112} However, some in vivo studies have been conducted in rats/mice where cancer was either induced (breast, hepatic, fibrosarcoma, adenocarcinoma)^{112–115} or transplanted (bladder, melanoma, mammary carcinoma).^{102,107,113}

Honey potentially inhibits the development of cancer by blocking the 3 main stages of carcinogenesis: initiation, proliferation, and progression. The reported mechanisms of its anti-proliferative, antimetastatic, and anticancer effects include

Table 2. Examples of immunostimulatory properties shown in vitro for honeys of diverse botanical and/or geographical origins.

HONEY (BOTANICAL/ GEOGRAPHICAL ORIGINS)	TYPE OF CELLS	EFFECTS	RESPONSIBLE COMPOUNDS (HYPOTHESIS)	REFERENCE
Not reported/Jordan	Peripheral blood B cells Peripheral blood T cells	↑ (either in the presence or absence of mitogens LPS, PHA, and Con A) ↑ (either in the presence or absence of mitogens LPS, PHA, and Con A)	Unidentified lymphommitogens	Abuharfeil et al ⁷⁷
Peroxide-generating honey from mixed floral source (pasture PS9)/not reported	They are not used	↑ production of hydroxyl radicals in the presence of Fe ²⁺ and other radical species	Production of other radical species may be due to secondary reactions between hydroxyl radicals and organic compounds present in the honey	Henriques et al ⁷³
Manuka honey with high levels of methylglyoxal (MGO250)/New Zealand	Neutrophils	↑ TNF-α only at lower concentrations (100 mg/mL)	Not referred	Chepulis and Francis ⁷⁴
Acacia/Slovakia	Primary keratinocytes	Upregulation of IL-1β, TGF-β, TNF-α, and MMP-9 mRNA expression	pH, hydrogen peroxide release and additional components	Majtan et al ⁶⁷
Acacia, buckwheat, manuka (UMF 15+)/Japan Buckwheat/Japan Buckwheat, acacia/Japan Manuka/Japan	HaCaT cells (human skin keratinocytes) HaCaT cells HaCaT cells HaCaT cells	Stimulation of MMP-9 expression Induction of syndecan-4 Upregulation of vimentin, HPRT-1, and STEAP-1 Upregulation of HPRT-1	Not referred	Ranzato et al ⁷⁵
Acacia, buckwheat/Japan	Human fibroblast cell line (46 BR.1N)	↑ release of IL-4, IL-6, and IL-8	Not referred	Ranzato et al ⁷⁸
Manuka honey (M61) and a mixed pasture honey (PS3)	Human monocytic cell line (MM6)	Induction of TNF-α release	Glycosylated proteins may affect MM6 activation	Tonks et al ⁶⁴
Manuka honey (airborne f/d), pasture honey (Lorimers)/New Zealand; Jelly Bush Honey/Austr. bush	Human monocytic cell line MM6 and human monocytes isolated from peripheral blood	↑ TNF-α, IL-1β, and IL-6 release	Component(s) that mediated the effects were unknown	Tonks et al ⁶⁵
Manuka honey/New Zealand	Mono Mac 6 cells (MM6) or murine bone marrow-derived macrophages from wild-type C57BL/6 mice or TLR2 or TLR4 knockout mice	Stimulation of the production of inflammatory cytokines TNF-α, IL-1β, or IL-6	Active component(s) of 5 to 6 kDa present in honey signal through TLR4 but not TLR2	Tonks et al ⁶⁶
Manuka honey UMF 16+/New Zealand), active 5+ Manuka honey/New Zealand), Danish honey	Human monocytic cell line Mono Mac 6 Human promyelocytic leukaemia cells HL-60	Induction of interleukin-6 release Release of reactive oxygen species	Heat stable substance with high molecular weight (>20 kDa)	Timm et al ⁷⁹
Kanuka (<i>Kunzea ericoides</i>), manuka (<i>Leptospermum scoparium</i>), and clover (<i>Trifolium</i> spp) honeys/New Zealand AGPs from kanuka honey	Human monocytic cell lines THP-1 and lymphoblast lung from human and U937	All 3 honeys stimulated TNF-α release from THP-1 cells, with kanuka honey being the most active AGPs purified from kanuka honey stimulated the release of TNF-α from THP-1 and U937 cells	Immunostimulatory properties may be attributed to their particular content of LPS, apalbumins, and AGPs	Gannabathula et al ⁶⁸
Thyme honey/France	Raw 264.7 murine macrophages	Increase in PGE ₂ production and overexpression of both COX-2 and TNF-α: overexpression and activation of the AP-1 and NF-κB transcription factor subunits	The induction of these pathways may depend on some unidentified components of thyme honey	Raynaud et al ⁶⁹

Abbreviations: AGPs, arabinogalactans; AP-1, activator protein 1; Con A, concanavalin A; COX-2, cyclooxygenase 2; HPRT-1, hypoxanthine phosphoribosyl transferase 1; IL, interleukin; LPS, lipopolysaccharide; MMP-9, matrix metalloproteinase 9; mRNA, messenger RNA; NF-κB, nuclear factor κB; PHA, phytohaemagglutinin; STEAP-1, six-transmembrane epithelial antigen of the prostate 1; TNF-α, tumour necrosis factor; TLR4, toll-like receptor; UMF, Unique Manuka Factor; ↑, increase; ↓, reduce.

induction of apoptosis, modulation of oxidative stress, cell cycle arrest, activation of mitochondrial pathways, induction of mitochondrial outer membrane permeabilization (MOMP), amelioration of inflammation, modulation of insulin signalling, modulation of oestrogen receptor activity, and inhibition of angiogenesis.¹¹⁶

Honey has been referred to as an apoptotic inducer through the upregulation and modulation of proapoptotic proteins (p53, Bax, caspase 3, and caspase 9) and downregulation of antiapoptotic proteins (B-cell lymphoma 2 protein [Bcl-2]). In addition, honey also produces ROS which will activate p53 that, in turn, modulates the expression of Bax and Bcl-2 and induces DNA fragmentation and activation of poly(adenosine diphosphate ribose) polymerase.¹¹⁶

Some honey proteins (royal jelly [RJ] proteins apalbumin 1 and apalbumin 2) can stimulate macrophages to release cytokines, such as TNF- α , IL-1 β , and IL-6. The production of TNF- α may be a key factor in this process. This cytokine is well known for regulating many cellular processes, including apoptosis, as it binds to the TNF receptor (TNFR), the TNFR-associated death domain protein, the TNFR-associated factor, and the receptor-interacting protein, hence stimulating both caspases 3 and 8.^{117,118}

Honey has antiproliferative activity by affecting cell cycle arrest and blocking the cell cycle of cancer cell lines (colon, glioma, and melanoma) in G₀/G₁, G₁, and G₂/M phases. The inhibition of cell proliferation induced by honey is due to the downregulation of several cellular pathways, via tyrosine cyclooxygenase, ornithine decarboxylase, and kinase.¹¹⁶

The antiproliferative activity of honey may also include the activation of the mitochondrial pathways and the release of proteins, such as cytochrome C, the induction of MOMP with the leakage of intermembrane space proteins moving into the cytosol causing cell death, and anti-inflammatory effect through the attenuation of pro-inflammatory mediators and inhibition of both NF- κ B and mitogen-activated protein kinase signalling pathways, which contribute considerably to the reduction in the second stage of cancer development (promotion).^{84,116,117,119}

These reported effects were dependent on the floral origin of the honey, as well as on the type of cancer cell. Along with the aforementioned RJ proteins, which are able to stimulate the release of cytokines, the anticancer/antiproliferative properties of honey may also be attributed to phenols (flavonoids and phenol acids) because it has established a significant correlation between these activities and the honey's phenol content.^{103,105,120,121} In addition, a positive correlation has been found between the anticancer activity and the amount of flavonoids (chrysin, apigenin, quercetin, acacetin, and pinocembrin) and phenolic acids (*p*-coumaric, vanillic, protocatechuic, caffeic, and *p*-hydroxybenzoic acids)^{103,105,116,117,119–122} present in honey, leading these authors to speculate about the anticancer potential of these secondary metabolites.

Metabolic and Cardiovascular Effects

Some metabolic alterations may increase the risk of cardiovascular diseases, namely, abdominal obesity, atherogenic dyslipidaemia, hypertension, insulin resistance, and glucose intolerance.¹²² Cardiovascular diseases are associated with chronic low-grade inflammation. Accordingly, if honey possesses anti-inflammatory activity, as discussed above, it may also play a role in the prevention of cardiovascular diseases, particularly if associated with other specific foods and dietary elements also beneficial to health, such as whole-grain foods and fresh fruit and vegetables.¹²³ Some studies report that when children consume honey and jam at breakfast, particularly older girls, the risk of cardiovascular diseases decreases.¹²² Moreover, González-Gil et al¹²³ report that moderate honey intake in European children decreases the probability of having higher concentrations of high-sensitivity C-reactive protein (CRP). Therefore, inflammation seems to be somehow associated with the type of diet.¹²³

In a 2008 study,¹²⁴ it was reported that natural honey ameliorates cardiovascular risk factors, both in healthy subjects and in patients with high risk factors. In both cases, natural honey reduced total cholesterol, low-density lipoprotein cholesterol (LDL-C), triacylglycerols, fasting blood glucose, and CRP and increased high-density lipoprotein cholesterol (HDL-C), with the advantage of not increasing body weight, particularly in overweight or obese individuals.

However, the proclaimed effects of honey on metabolic and cardiovascular systems are still controversial. For example, in healthy humans, Al-Waili¹²⁵ observed that honey reduced cholesterol, LDL-C, triglycerides (TG), homocysteine, and CRP and slightly elevated HDL-C. In addition, they observed that in patients with hypertriglyceridaemia, honey decreased TG; in patients with hyperlipidaemia, honey decreased LDL-C, and in patients with diabetes, honey decreased the levels of glucose in the plasma. Such observations support the positive role of honey in the prevention of cardiovascular diseases. Conversely, other publications show that honey consumption increases the levels of glycosylated haemoglobin in patients with diabetes, suggesting that it does not help with the glycaemic control in these patients.¹²⁶ These findings were further investigated by a different group of authors¹²⁷ who argue that those results pertain to the administration of high doses of honey. In fact, this publication shows that low doses of honey, irrespective of its geographical origin,^{128,129} improve hyperglycaemia and dyslipidaemia in alloxan-induced diabetic rats. These properties are most likely derived from the fructose and other oligosaccharides present in honey.^{129,130} In fact, glucose and fructose have a synergistic effect in the gastrointestinal tract by enhancing intestinal fructose absorption and/or stimulation of secretion of insulin. At the same time, fructose improves the hepatic glucose uptake and glycogen synthesis and storage, activating the hepatic glucokinase and glycogen synthase.¹²⁹

It has been proposed that honey mimics some characteristic effects of diverse enzyme inhibitors, namely, α -glucosidase inhibitors, insulin secretagogues, thiazolidinediones and biguanides, dipeptidyl peptidase-4 inhibitors, and anti-obesity drugs.¹³¹ The putative antidiabetic benefits of honey have been demonstrated in vivo using laboratory model organisms. However, in humans, the studies demonstrating the beneficial effects of this natural bee product, particularly regarding the lowering of glucose in the blood, are still insufficient to draw reliable conclusions, mainly because the assays lasted only a few weeks (8-12 weeks). Because diabetes is a chronic disorder, such a limited amount of time is not enough for categorical answers regarding the antidiabetic potential of honey.¹³² Additional constraints that hamper the objective study of honey's antidiabetic effect are the small size of the samples, absence of randomized clinical trials where patients with diabetes are grouped by disease severity (mild, moderate, and severe), treatment with different dosages of honey, as well as the absence of continued clinical studies concerning the short-term and long-term effects of honey consumption in patients with diabetes.¹³¹

Hypertension is another important public health disease, not only because of its high prevalence but also because of its another critical risk factor for the development of cardiovascular diseases.¹³³ Some authors demonstrated that honey was able to reduce elevated systolic blood pressure in spontaneously hypertensive rats. At the same time, the honey supplement was also able to improve the oxidative stress observed in the kidneys of those experimental animals through the decrease in malondialdehyde levels.¹³⁴

The metabolic and cardiovascular effects of honey are highly dependent on its chemical composition, akin to its other biological effects. Quercetin and kaempferol (flavonoids present in honey, whose concentration depends on its floral/geographical origin) and their metabolites may have a protective effect on cardiovascular disease via several reported mechanisms: (1) activation of Ca^{2+} -activated K^+ channels and increasing the activity of endothelial NO synthase with stimulation of arterial relaxation, (2) protection of vessels against hypochlorous acid-induced endothelial dysfunction in isolated arteries by the activation of adenosine monophosphate protein kinase and subsequent increase in NO production, and (3) suppression of TNF- α production as well as activation of NF- κ B, with the consequent inhibitory activity on cell adhesion and migration to endothelial cells.^{59,135-139} These properties attributed to flavonoids and their derivatives have mostly been demonstrated in cell cultures or in laboratory animals. However, other compounds alone or, most likely, in association may also play a decisive role in the achievement of the beneficial properties attributed to honey.

Prebiotic Properties

Prebiotics are non-digestible food ingredients with functional effects on the gastrointestinal tract, which are usually short-chain carbohydrate compounds, primarily oligosaccharides. They stimulate the growth of bifidobacteria and lactobacilli populations in the colon, being selectively fermented by them. These events increase the production of short-chain fatty acids, decrease the pH, and reduce the fat absorption and ammonia production, overall enhancing the host's health.^{140,141}

Fructooligosaccharides, galactooligosaccharides, and inulin, which are present in some natural foods, are examples of prebiotics,^{142,143} whose degree of polymerization and type of oligomer, which differ from food to food, are responsible for much of their effectiveness.¹⁴⁰ Honey, having circa 0.75% of fructooligosaccharides in its constitution,^{144,145} may act as a prebiotic. Moreover, the detrimental effects of bile salts on *Bifidobacterium* spp, a ubiquitous inhabitant of the human gastrointestinal tract, can be overcome by the action of fructooligosaccharides and their monomeric derivatives.¹⁴⁶ The prebiotic effect of honey has been observed in several monofloral honeys: sourwood, alfalfa and sage honey,¹⁴⁷ honeydew honey,¹⁴⁸ chestnut and acacia honey,¹⁴⁹ clover,¹⁵⁰ and eucalyptus,¹⁵¹ each with strengths highly dependent on its floral origin.^{147,149} In addition, some authors observed that the prebiotic activity of honey was not comparable with the one demonstrated by inulin or fructooligosaccharides, being substantially inferior,^{141,148} acting preferentially on *Bifidobacterium*,¹⁵² or otherwise.^{148,153}

Control of Human Pathogens With Honey

Since ancient times, honey has been used to control infections. Nowadays, this practice has seen a renewed interest in virtue of the emergence of antibiotic-resistant bacterial pathogens, which increase the risk of simple medical interventions. The antibacterial features of honey have been linked to different factors, including the amount of hydrogen peroxide (H_2O_2), low pH, high osmolarity, phenolic and aromatic acid content, honey glycoproteins, as well as products of the Maillard reaction (this non-enzymatic browning reaction involves the interaction between reducing carbohydrates and amino acids or proteins).¹⁵⁴⁻¹⁵⁸ A recent publication demonstrated the antibacterial activity of the MRJP1 honey glycoprotein against bacterial clinical isolates.¹⁵⁷ However, these findings were not reproduced by the subsequent study conducted by Bucekova and Majtan¹⁵⁹ that tested the action of the purified MRJP1 honey glycoprotein isolated from 3 honey types, which are used in wound healing (manuka honey, Revamil source honey, and honeydew honey). The discordant results between the 2 studies may, however, be related to the differences in the methodology used to isolate and purify the honey's glycoproteins.

The antibacterial activity of the different honeys has been shown to be linked to its geographical origin, botanical origin,

season of harvest, and conditions of processing and storage.^{160,161} Despite the recognition of the valuable action of honey as an antibacterial agent, its mechanism of action against pathogens is still poorly understood. However, there is an increase in the number of studies aiming at investigating the interactions between honey and bacterial cells, particularly with manuka honey, which is one of the most effective honeys.^{161–166} Those studies show that, depending of the bacterial species, the honey's action influences the bacterial size (shorter or longer cells), morphology, cell division (incomplete division), surface (irregular), motility, injury to nucleic acids, and lysis.

Bacterial pathogens in nature typically live in biofilms, ie, adherent to biotic or abiotic surfaces. Of particular concern for the human health is the fact that bacteria adhere to medical devices and colonize the mucosal membranes, as well as the skin and open wounds. The ability to adhere and maintain a cohesive biofilm structure is mainly regulated by a cellular communication process denominated quorum sensing (QS). Several studies have explored the potential of honey for the impairment of the natural ability of bacteria to adhere and form a biofilm, disrupt an already established one, and even inhibit the QS process.^{166–171}

Disruption of QS and biofilm

Quorum sensing is a cell-cell communication system that involves the production of signalling molecules, such as *N*-acyl-L-homoserine lactones (AHLs), which control cellular processes linked to population density, including biofilm formation and virulence.^{172,173}

In the study of Truchado et al,¹⁶⁷ chestnut honey (*Castanea sativa*) and its aqueous extract inhibited the QS of 2 common biosensor bacteria, namely, *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* NTJ, and its QS inhibitory ability was additionally confirmed in 3 relevant pathogens: *Erwinia carotovora*, *Yersinia enterocolitica*, and *Aeromonas hydrophila*. The QS inhibitory features of chestnut honey were associated with both inhibition of AHL production and its degradation. Simultaneously, pathogen growth was not affected, but biofilm formation, which is monitored by QS, was impaired.

The QS inhibitory potential of manuka honey was identified either via the testing of the QS system in *Pseudomonas aeruginosa*⁶⁵ or by evaluating the biosensor of *C violaceum*.¹⁷¹ Both QS systems are crucial for these pathogens to overcome environmental stresses and adequately adjust their virulence state. Both manuka and Italian honeys interfere with the 2 QS systems of *P aeruginosa*. Interestingly, the authors show that the QS inhibition was associated with the honey's sugar content because the expression of the *pqsA* gene was nearly 50% reduced at a concentration of 1% of honey (both manuka and Italian). Moreover, the bacterial growth was not inhibited for up to 6% concentrations of either manuka or Italian honey.¹⁶⁹ The effect

of honey's sugar content on the inhibition of QS has also been reported by Lee et al,¹⁶⁸ who tested the effect of diluted honey (a monofloral [acacia] and a polyfloral Korean honey) on the QS system of the enterohemorrhagic *Escherichia coli* 0157:H7. The authors suggested that certain sugar concentrations might block *E coli*'s AHLs from entering the cell and for that reason prevent AHL ligation to its receptor.

Slovakian honey (hawthorn [*Crataegus laevigata*], honeydew [*Abies alba* Mill], and acacia [*Robinia pseudoacacia*]) and also manuka honey could prevent biofilm formation by the wound-associated bacteria *Proteus mirabilis* and *Enterobacter cloacae*. At 50%, these honeys display the potential to induce the detachment of bacterial cells from aggregates.¹⁷⁴ However, none of the tested honeys has shown the ability to break up *E cloacae*'s biofilm.¹⁷⁴ Another interesting study, using a modified Lubbock chronic wound biofilm system, examined the effect of honey and a recombinant form of the honey defensin 1 (Def-1) against a multispecies biofilm (*Staphylococcus aureus*, *Streptococcus agalactiae*, *P aeruginosa*, and *Enterococcus faecalis*).^{175,176} These authors found that the pathogens *S aureus*, *S agalactiae*, and *P aeruginosa* were indeed perturbed by the tested honeys (manuka and honeydew). Defensin 1 affected *S aureus* and *P aeruginosa* but not *S agalactiae*. Interestingly, *E faecalis* sessile cells were resistant to both honey and Def-1.

The anti-biofilm activity of honey has been associated with different substances, either alone or in combination, namely, with sugars, phenols, hydrogen peroxide, dicarbonyl methylglyoxal, and even its low pH and high osmolarity.^{169,175}

The effect of honey on biofilm inhibition can be improved if honey is associated with antibiotics. Campeau and Patel¹⁷⁷ tested manuka honey together with vancomycin, showing a synergistic activity of this association against the biofilms of *S aureus* (the minimum biofilm eradication concentration [MBEC] of manuka honey used alone was 3% and with 1 µg/mL vancomycin was 1%), whereas the association of manuka with gentamycin showed an additive activity against *P aeruginosa* biofilm (the MBEC value of manuka honey used alone was 30%, with 75 µg/mL of gentamycin was 20%, and when associated with 100 µg/mL of gentamycin dropped to 17.5%).

Antivirulence action

Antivirulence agents (AVAs) impair virulence factors (VFs) by negatively affecting their production/activity, constituting a supplementary or alternative approach to combat the emerging resistant pathogens. This activity is associated with the fact that AVAs do not disturb the bacterial growth, hence not exerting the environmental pressure that classical antimicrobials do, which lead to a positive selection of the genetic mutations that allow the resistant cells to subsist. Honey offers the possibility of acting as a natural anti-VF. Inside the host, some pathogens experience iron deficiency, and to overcome this, they depend

on siderophores to acquire iron. As such, inhibition of siderophore production constitutes an alternative means of controlling pathogens. In the study by Kronida et al,¹⁷⁸ it was observed that manuka honey impairs *P aeruginosa*'s virulence by interfering with siderophore production.

Using a combined proteomics and transcriptomics approach, Jenkins et al¹⁷⁰ observed that manuka honey affected the expression of VFs of methicillin-resistant *Staphylococcus aureus* (MRSA), namely, the expression of the virulence genes *sec3*, *fnb*, *hlgA*, *lip*, and *hla*. However, its real impact in vivo was not demonstrated.

The number of case reports about the usage of honey on wounds and ulcers resilient to healing is very limited.^{179,180} However, the published data pointed out to a considerable enhancement of infection clearing after unsuccessful antibiotic treatment.^{179,180}

Using animal models to study infection and wound healing is not consensual. Nevertheless, the impact of honey on the virulence potential should be clearly evaluated using, eg, insect models, such as the greater wax moth (*Galleria mellonella*), whose innate immune response shows significant similarity with the vertebrate immune response.¹⁸¹ In a recent study, da Silva et al¹⁸⁶ have shown that Portuguese honeys were able to impair the virulence potential of *S aureus* and MRSA strains, using the *G mellonella* insect model. The potential action of honey against *S aureus* is timely and pertinent as this pathogen is one of the main sources of infection associated with health care institutions.¹⁸²

The usage of honey in combination with other antimicrobial agents

The usage of honey in combination with other antimicrobial agents has been the subject of some investigations.^{183–185} Combining honey with other antimicrobial agents diminishes the chances of pathogen survival after exposure, consequently reducing the likelihood of emergence of resistant bacteria. The usage of manuka honey, in combination with the antibiotics tobramycin and colistin, to combat *P aeruginosa* and *Burkholderia cepacia* infections resulted in synergistic or additive effects against the 2 strains of each bacterial species.¹⁸⁴

Furthermore, the combination of manuka honey with rifampicin, oxacillin, gentamycin, and clindamycin was tested against different MRSA strains and methicillin-susceptible *Staphylococcus aureus* clinical isolates, finding that, depending on the strain, it yielded synergistic or additive effects or even just increased the susceptibility of the bacterial isolates to the antibiotic.¹⁸⁵

As mentioned above, the combination of honey with antibiotics has been shown to improve the inhibition of biofilm formation.¹⁷⁸

An interesting report demonstrated that the action of 2 honeys collected from the stingless bees *Scaptotrigona*

bipunctata (Lepeletier, 1836) and *Scaptotrigona postica* (Latreille, 1807), when in combination, shows a synergistic type of action against several bacterial pathogens, including multiresistant bacteria.¹⁸⁶ It is possible that such synergistic action arises from the combination of the constituents of the 2 honeys. Other studies have also reported synergistic effects between bee products, particularly the combination of honey with propolis, against individual or polybacterial resistant pathogens.¹⁸⁷ Another intriguing synergistic effect has been found with the combination of honey with potato starch.¹⁸⁸ This combination was shown to be very effective against the bacterium *Klebsiella pneumoniae* and was correlated with the content of fructose, glucose, and diastase in the mixture.

Antiviral Activity of Honey

Studies examining the antiviral activity of honey are limited. The manuka honey, one of the best studied honey types, revealed to be effective against influenza virus in vitro, using Madin-Darby canine kidney cells.¹⁸⁹ Manuka and clover honey were also effective in vitro against varicella zoster virus using human malignant melanoma cells (MeWo).¹⁹⁰ Furthermore, commercial and manuka honey seem to act against herpes simplex virus (HSV-1) isolates in vitro (using Vero cells),^{191,192} and honey solutions act against rubella virus also in vitro (using monkey kidney cell cultures).¹⁹³ Al-Waili¹⁹⁴ showed that topical honey application was effective in healing labial and genital recurrent herpes lesions. The report discusses that the amelioration of symptoms and signs of herpetic lesions by honey might be attributed to the inhibition of prostaglandins at the lesion site.

In these studies, the antiviral activity has not been attributed to any particular compound, or group of compounds, present in honey. Viuda-Martos et al,³ however, consider that flavonoids may have an important role for this activity because galangin has been proven to be effective against HSV and coxsackie B virus, whereas quercetin and rutin show antiviral activity against HSV, syncytial virus, poliovirus, and Sindbis virus. Although these flavonoids may be found in honey, an unequivocal connection between the antiviral properties of honey and its chemical composition has not yet been formally established.

Disadvantages

Despite the limited evidence performed in the clinical environment to thoroughly compare the risk/benefits of using honey versus the conventional treatments, it is undisputed that honey is more and more used, not only as a food source but also for alleviating particular ailments.

Regardless of its usage as food or remedy, honey may be contaminated by pesticides, antibiotics, heavy metals, and other toxic compounds. The presence of such compounds may be attributed not only to accidental exposure and environmental hazards but also to compounds added by beekeepers to control honeybee

diseases.¹⁹⁵ However, these contaminants may be harmful for human health, producing unexpected consequences.

In addition to those chemical compounds, honey may also be contaminated with pathogens, particularly *Clostridium botulinum* and its spores. As such, the consumption of honey or its derivatives is considered to be dangerous for infants, the elderly, and immunocompromised individuals.¹⁹⁶ For this reason, honey used for therapeutic purposes should be sterilized using gamma irradiation.¹⁹⁵

For external usage, honey may also present some disadvantages or adverse effects: preparation of impregnated dressings may be difficult; it becomes more fluid at high temperatures, potentially liquefying at wound temperature; transient stinging sensation may occur when honey is applied topically; topical application in large wounded areas of patients with diabetes may increase the concentration of glucose in the blood up to dangerous levels; excessive application of honey may lead to tissue dehydration; and allergic reaction to pollen or to bee proteins in the honey may occur.^{197–199}

Conclusions

Despite the abundant potentially bioactive properties reported above, a comprehensive overview of the mechanisms underlying honey's health benefits is still under active scrutiny. The studies conducted so far support that each honey's activity is strongly dependent on its floral or geographic origin. The most investigated properties of honey for its potential usage as an alternative medicine include (1) its antioxidant activity, (2) its ability to induce anti-inflammatory and immunomodulatory responses, (3) delaying cancer development and cardiovascular diseases, (4) inhibiting bacterial pathogens by interfering with their pathogenesis potential, (5) its ability to control viral infection, and, finally, (6) improving the health of the gut by acting as a prebiotic agent.

The observed synergistic actions between honey and some antibiotics highlight the need for the adjustment of antibiotic dosage for those patients who are using honey as a natural supplement. Exploring the synergistic effects of honey with other bee products, or vegetable products, seems also to be another promising venue of investigation. In addition, pinpointing the honey's chemical constituents responsible for its reported antiviral properties is also a remarkable feature that requires further research. In addition, there is a pressing need to chemically characterize the different types of honey, particularly regarding their geographical and floral origin, to create a catalogue of beneficial compounds that are present in each honey type, allowing the end users to choose the type of honey that best fits their needs.

Altogether, there are various pieces of evidence reported in numerous comprehensive studies, clearly demonstrating that honey constitutes an efficient nutraceutical agent, potentially operating on several vital health systems.

Author Contributions

Conceived the concept: MGM, MDA, MLF. Wrote the first draft of the manuscript: MGM, MDA, MLF. Contributed to the writing of the manuscript: MGM, MDA, MLF. Agreed with manuscript results and conclusions: MGM, MDA, MLF. Made critical revisions and approved the final version: MGM, MDA, MLF. All authors reviewed and approved the final manuscript.

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