Jibril et al. Bulletin of the National Research Centre (2019) 43:4
https://doi.org/10.1186/s42269-019-0044-7

Bulletin of the National Research Centre

REVIEW Open Access

Isolation and characterization of polyphenols in natural honey for the treatment of human diseases

Fatima Ibrahim Jibril[1], Abu Bakar Mohd Hilmi[1*] and Lavaniya Manivannan[2]

Abstract

Honey is a natural sweetener that is derived from the nectar, pollen, and resin of plants. It has been used as a folk medicine for decades. In addition to being an excellent therapeutic agent, honey possesses an unusually high nutritional content, thus generating interest among researchers. The major phytonutrients of honey are polyphenols. Polyphenols can be separated using high-performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS). Moreover, to separate the volatile compounds in honey, gas chromatography-mass spectrometry (GC-MS) may be used. Polyphenols have unique and complex structures that are mainly composed of flavonoids and phenolic acids, which confer significant antiviral, anti-inflammatory, antineoplastic, and antiulcer effects and can be used to treat chronic diseases, such as cardiovascular disease. The nature and variability of polyphenols in different honeys posed a challenge to investigations in previous years. Nevertheless, the significant role of honey as a natural therapy and its enrichment with natural substances have led to the continuous discovery of efficient, reliable, and rapid methods for the identification and quantification of novel bioactive compounds in honey. This current review highlights the above mentioned issues.

Keywords: Monofloral and multifloral honey, Bioactive compound, Polyphenols, Flavonoid, Phenolic acid

Introduction

Honey is a natural sweetener that is produced by an insect, the bee, from the family Apidae. Since ancient times, it was used among Arabic, Egyptian, and Indian gentility. At that time, honey use was not limited to food, but it was also used as a royal gift or drink, as a sweetener or preservative, and as a home treatment for family members who were suffering from skin rashes, wounds, cough, or sore throat (Molan 1999; Molan 2006; Allsop and Miller 1996). Sweet honey contains phytochemical substances that are obtained when a bee sucks the nectar of flowers or secretions from living parts of plants, such as resin. Resin is found in immature fruit and flower buds. The pollen in the honey is an identifying marker for the botanical origin of the honey. Phytochemical substances are then carried by bees in their honey sacs and stored in honeycombs until the time of harvest. During this process, the natural phytochemical substances are mixed with fluid from the bee's body, thereby producing bioactive compounds with different structural components that are present in honey. Bioactive compounds possess substantial biological activity (Nile and Park 2014) and display unique abilities to elicit responses (Ramirez-Estrada et al. 2016) in living organisms and to improve health care (Rodriguez-Mateos et al. 2014). Bioactive compounds not only vary from each other with respect to their characteristics and the magnitude of the effects they elicit, but they also differ functionally (Brusotti et al. 2014; Forbes-Hernández et al. 2014). The efficacy of bioactive compounds for the treatment of human diseases has led to extensive profiling studies of the bioactive compounds present in honey or in herbal plants. The aim of this review is to highlight the isolation and characterization of natural bioactive compounds in honey and to explore the applications of polyphenols for the treatment of human diseases.

* Correspondence: mhilmiab@unisza.edu.my
[1]School of Biomedicine, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, 21300 Kuala Nerus, Terengganu, Malaysia
Full list of author information is available at the end of the article

Diversity of honey

More than 200 polyphenol compounds have been identified in various honey samples (Eteraf-Oskouei and Najafi 2013; Gheldof et al. 2002). For their nutrition, honeybees depend intensely on their ability to forage for aqueous solutions in nearby plants (Ball 2007; Seeley 2009). Most of the plant fluids they consume, such as nectar or resin, are mainly composed of protein, different types of sugars, lipids, minerals, vitamins, and ascorbic acid (Baker and Baker 1983). The composition of such fluids varies depending on the species of plant and natural geographical features (Graham 1992). Thus the composition of a given honey sample depends on its source (Schmidt 1996). Commonly, bees from the genus *Apis* forage on a single floral source, thus producing monofloral honey. Most honeys are monofloral and are named according to their respective plant species. Manuka honey, the well-known New Zealand and Australian medical-grade honey, is named after the manuka tree, *Leptospermum scoparium*. Similarly, alfalfa honey, avocado honey, sage honey, and tualang honey are named after their respective source trees. In contrast, bees from the genus *Trigona*, known as stingless honeybees, forage on diverse floral sources and produce polyfloral or multifloral honey. As such honey cannot be named after a tree species, multifloral honey is named according to the forager bee species, such as *Trigona itama* honey or *Trigona thoracica* honey. Nevertheless, the relative efficacy of monofloral and multifloral honeys for the treatment of human disease remains under investigation, as does a conclusion about which type of honey represents a superior therapeutic product.

The most abundant constituents of honey are carbohydrates, which include monosaccharides, disaccharides, and polysaccharides (75%); water (20%); proteins and free amino acids (2%); acids (1%); minerals (1%); and vitamins and enzymes (1%) (Krell 1996). It is a formidable challenge to define a standard honey for human use, due to the great variation in honey compositions and geographical origins (McLoone et al. 2016). For example, the honeys derived from the Apini tribe (non-stingless bee) and the Meliponini tribe (stingless bee) are composed of complex mixtures of natural phytochemical substances; to date, their vital novel compounds that promote human health and consumption remain under investigation.

Honey profiling

Honeys made by the genera *Apis* and *Trigona* are both composed of major and minor phytochemical compounds that can be characterized using various analytical methods. The characterization of phytochemical compounds begins with the preparation of honey samples and is followed by extraction and finally by separation or quantification (Khoddami et al. 2013). Prior to each profiling analysis, the preparation of representative samples requires both a sufficient quantity of honey that is free of contaminants and an accurate solution concentration to verify the quantitative and qualitative determinations. Unnecessary analytical repetitions due to errors are time-consuming and can be effectively avoided if sample preparation is precise and optimized. Homogenizing the sample manually or mechanically (Reybroeck et al. 2010; Kurhade et al. 2013), with mild heating to reduce crystallization (Aljadi and Yusoff 2003), is another key procedure for obtaining accurate chromatography results.

The minor phytochemical compounds in honey can be easily isolated by extraction techniques. To increase the concentrations of these minor compounds in a representative sample of honey, sugar and matrix compounds are first extracted by using organic solvents. This procedure may result in uniformly enriched target compounds and less interference from the matrix compound (Tura and Robards 2002). In addition, the use of other solvents, such as ethyl acetate (Kassim et al. 2010a; Kassim et al. 2010b), ethanol (Brudzynski and Miotto 2011; Alvarez-Suarez et al. 2010), methanol (Brudzynski et al. 2012), acetone (Naqvi et al. 2013), water, or mixtures of these solvents (Routray and Orsat 2012; Macdonald et al. 2010) contribute to the optimal extraction of target compounds, especially minor polyphenol substances. Solvents are selected based on the intended analytical technique and on the molecular weight, molecular size, solubility, pH, and volatility of the target compound. An acidic compound is isolated by using a compatible acidic organic solvent. A 70% ethanol solution is suitable for microwave-assisted extraction, ultrasonic extraction, and maceration extraction. On the other hand, ethyl acetate is recommended for liquid-liquid extraction. Multiple extraction methods are used for the isolation of polyphenols; they include supercritical fluid extraction (Junior et al. 2010), pressurized liquid extraction (Blasco et al. 2011), stir-bar sorptive extraction (Huang et al. 2011), and solid-phase micro extraction (Campillo et al. 2012).

Ion-exchange column chromatography is the most common assay for the characterization of minor phytochemical compounds derived from natural honey. Each phytochemical compound is detected based on its ultraviolet absorption peak, its molecular weight, or its retention time. Liquid chromatography-based separation techniques, such as liquid chromatography-mass spectrometry (LC-MS) and high-performance liquid chromatography (HPLC), are the preferred methods for the characterization of polyphenol compounds. Recently, HPLC and GC have been combined with MS to establish HPLC-MS and gas chromatography-mass spectrometry (GC-MS) techniques (Biesaga and Pyrzyńska 2013). Of

all these techniques, HPLC and GC-MS are most commonly used (Merken and Beecher 2000; Sun et al. 2012) because of their reliability, efficiency, accuracy, and speed. Generally, GC-MS is suitable for the characterization of phenolic acids and other volatile compounds. Meanwhile, LC-MS and HPLC are useful for the characterization of flavonoids and other major compounds. Separation by LC-MS includes ultraviolet detection with a photodiode detector and diverse subsequent mass-spectrometric methods (Biesaga and Pyrzyńska 2013; Pyrzynska and Biesaga 2009). However, such techniques are not only time-consuming but also tedious and costly. Other techniques, such as ultra-high-performance liquid chromatography (UHPLC) (Kečkeš et al. 2013; Gašić et al. 2014), HPLC with diode-array detection (HPLC-DAD) (Mattonai et al. 2016), reverse-phase HPLC (Can et al. 2015), paper chromatography, thin-layer chromatography (Naczk and Shahidi 2006), and capillary electrophoresis (Asensio-Ramos et al. 2009) are among the analytical choices for minor and major phytochemical profiling. The quantification of bioactive compounds is an optional analysis that is performed by comparing a known commercial standard with each separated compound. Table 1 shows the characterization of polyphenols as verified by different chromatography techniques.

Polyphenols

Natural honey is composed of nutritive polyphenols (Manyi-Loh et al. 2011). Polyphenols are primary identifying markers for the botanical origin of honey (Wang and Li 2011), and they display high therapeutic and dietary value (Uthurry et al. 2011). Bioactive polyphenol compounds are responsible for the colors, aromas, and tastes of all plant-based products, including fruits, cereals, and vegetables (Chaturvedula and Prakash 2011). The different polyphenol-derived ranges of honey color, aroma, and taste are directly dependent on the pollen source (Gil et al. 1995). More importantly, polyphenols integrate the physiological functions of a plant and are composed of secondary metabolites of various groups or classes (Di Ferdinando et al. 2014). Specific examples of such metabolites include tocopherol, flavonoids, phenolic acids, alkaloids, chlorophyll substances, amino acids (peptides), amines, carotenoid derivatives, and ascorbic acid (Cavalcanti et al. 2013). The phenolic groups of polyphenols derived from citrus fruits are vital for reducing proinflammatory cytokines in humans (Morand et al. 2011; Bernabé et al. 2013).

Flavonoids and phenolic acids

The major polyphenol compounds in honey are flavonoids and phenolic acid (Khalil et al. 2011). Flavonoids and phenolic acid are responsible for inhibiting oxidation and destroying free radicals (Nakajima et al. 2014). The identification and classification of flavonoids and phenolic acid are based on their chemical structures, which consist of one or more hydroxyl groups that are fused with an enclosed ring structure and thereby produce an aromatic ring containing six carbon atoms with hydrogen atoms (Harborne 1989).

The identities of the bioactive flavonoid and phenolic acid compounds in honey are similar across different types of honey but vary in their relative quantities (Can et al. 2015). Table 2 shows the bioactive flavonoid and phenolic acid compounds found in various types of honey. Honey of darker color contains higher quantities of bioactive compounds than lighter-colored honey (Moniruzzaman et al. 2013). This difference is due to the nutritional differences among different sources of pollen (Estevinho et al. 2008; Estevinho et al. 2012).

Polyphenols in natural honey for the treatment of human diseases

Antimicrobial activity

The presence of flavonoids and phenolic acids confers substantial antimicrobial activity to honey. With respect to the antibacterial effects of honey, previous studies revealed the activity of extracted esters of flavonoids and phenolic acids against various bacterial species, including *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus lentus*, *Klebsiella pneumoniae*, and *Escherichia coli* (Estevinho et al. 2008). Specifically, the inhibitory activities of apigenin, quercetin, myricetin, and rutin against *E. coli*, *Vibrio cholerae*, *Salmonella typhi*, and *Salmonella typhimurium* have been reported (Das et al. 2015). Similarly, the high antibacterial activity of naringenin against streptococci and methicillin-resistant *Staphylococcus aureus* has also been reported (Tsuchiya and Iinuma 2000). Additionally, myricetin has been described as an inhibitor of DNA synthesis in *Proteus vulgaris* (Mori et al. 1987; Tenore et al. 2012).

Honey also has shown antiviral effects, as it was found to be effective in the treatment of labial and genital herpes, which are common viral diseases (Onifade et al. 2013; Fingleton et al. 2014). Another study demonstrated the direct effects of several flavonoids, such as chrysin, acacetin, and flavones, as potent inhibitors of HIV replication, which severely infects H9 lymphocytes (Critchfield et al. 1996; Behbahani 2014). Chrysin reportedly inhibits herpes simplex type-1 and type-2 virus in addition to adenovirus-3 (Lyu et al. 2005). Furthermore, apigenin was characterized as an antiviral against hepatitis B surface antigen and hepatitis B e-antigen (Chiang et al. 2005).

Antioxidant activity

Antioxidants are natural chemical substances that are mainly found in plants. Their main role is as a defense

Table 1 Characterization of polyphenols as verified by different chromatography techniques

Polyphenols	Honey samples	Eluents	Chromatography techniques
Flavonoids			
Apigenin	Chesnutt	Methanol	HPLC (Can et al. 2015)
Catechin	Heather	Methanol	HPLC (Can et al. 2015)
Catechol	Stingless bee	Methanol, ethyl acetate	HPLC (da Silva et al. 2013)
Chrysin	Lime	Methanol	GC-MS (Lachman et al. 2010)
Dihydrochrysin	Lime	Methanol	GC-MS (Lachman et al. 2010)
Galangin	Lime	Methanol	GC-MS (Lachman et al. 2010)
Hesperetin	Gelam	Methanol	HPLC, LC-MS (Kassim et al. 2010b)
Isorhamnetin	Acacia	Methanol	HPLC (Can et al. 2015)
Kaempferide	Gelam	Methanol	LC-MS (Kassim et al. 2010b)
Kaempferol	Gelam	Methanol	HPLC (Kassim et al. 2010b)
Luteolin	Stingless bee	Methanol, ethyl acetate	HPLC (da Silva et al. 2013)
Myricetin	Gelam	Methanol	HPLC (Kassim et al. 2010b)
Naringenin	Stingless bee	Methanol, ethyl acetate	HPLC (da Silva et al. 2013)
Pinobanksin	Buckwheat	Ethanol, methanol, isopropanol	LC-MS (Alvarez 2011)
Pinocembrin	Buckwheat	Ethanol, methanol, isopropanol	LC-MS (Alvarez 2011)
Pinostrobin	Buckwheat	Ethanol, methanol, isopropanol	LC-MS (Alvarez 2011) HPLC, LC-MS (Alvarez 2011),
Quercetin	Gelam	Methanol	LC-MS (Kassim et al. 2010b)
Quercetin-3-O-glucoside	Gelam	Methanol	LC-MS (Kassim et al. 2010b)
Rhamnosyl naringenin	Gelam	Methanol	HPLC (Kassim et al. 2010b)
Rutin	Lavender	Methanol	GC-MS (Can et al. 2015)
Taxifolin	Rape	Methanol	GC-MS (Lachman et al. 2010)
Tectochtysin	Rape	Methanol	GC-MS (Lachman et al. 2010)
Phenolic acids			
Abscisic acid	Rape	Methanol	HPLC (Lachman et al. 2010)
Benzoic acid	Gelam	Methanol	HPLC (Kassim et al. 2010b)
Caffeic acid	Gelam	Methanol	HPLC (Kassim et al. 2010b)
Chlorogenic acid	Stingless bee	Methanol, ethyl acetate	GC-MS (da Silva et al. 2013)
Cinnamic acid	Lime	Methanol	HPLC, LC-MS (Lachman et al. 2010)
Dihydroxycinnamic acid	Gelam	Methanol	LC-MS (Kassim et al. 2010b)
Ellagic acid	Gelam	Methanol	LC-MS (Kassim et al. 2010b)
Ellagic-glucoside	Gelam	Methanol	GC-MS (Kassim et al. 2010b)
Ellagitannins	Rape	Methanol	HPLC (Lachman et al. 2010)
Ferulic acid	Rape	Methanol, ethyl acetate	HPLC, LC-MS (da Silva et al. 2013)
Gallic acid	Stingless bee	Methanol	HPLC (Kassim et al. 2010b)
p-Coumaric acid	Gelam	Methanol	HPLC (Can et al. 2015)
Protocatechuic acid	Chesnutt	Methanol, ethyl acetate	LC-MS (Kassim et al. 2010b)
Salicylic acid	Stingless bee	Methanol	HPLC (da Silva et al. 2013)
Syringic acid	Stingless bee	Methanol	HPLC (da Silva et al. 2013)
Vanillic acid	Stingless bee	Methanol	LC-MS (Kassim et al. 2010b)

mechanism that counteracts free radicals and prevents their damaging oxidative effects (Manyi-Loh et al. 2011; Kumar et al. 2013). Oxidative stress can lead to pathogenesis or to the mutation of cellular DNA. Moreover, free radicals have roles in cardiovascular disease, diabetes, cancer, aging, gastritis, Alzheimer's disease, and

Table 2 The bioactive flavonoid and phenolic acid compounds found in honey

Honey origin	Polyphenols	Honey type
Brazil	Naringenin, quercetin, isorhamnetin, gallic acid, vanillic acid, 3,4-dihydroxybenzoic acid, coumaric acid	Stingless bee honey (da Silva et al. 2013)
Bulgaria	Gallic acid, ferulic acid, caffeic acid, p-coumaric acid, 4-hydroxybenzoic acid, vanillic acid, syringic acid, protocatechuic acid	Eucalyptus, lime, chestnut, heather, lavender, acacia, rosemary, orange, sunflower, and rapeseed honey (Dimitrova et al. 2007)
Malaysia	Gallic acid, syringic acid, caffeic acid, vanillic acid, p-coumaric acid, benzoic acid, naringenin, trans-cinnamic acid, luteolin	Tualang, Gelam, Borneo tropical honey (Khalil et al. 2011)
New Zealand	Gallic acid, caffeic acid, ellagic acid, quercetin, isorhamnetin, chrysin, luteolin, kaempferol	Manuka honey (Yao et al. 2004)
Poland	Rhamnetin, naringenin, quercetin, rutin, hesperidin, vanillic acid, p-coumaric acid, chlorogenic acid, protocatechuic acid, syringic acid.=	Heather honey (Biesaga and Pyrzyńska 2013)
West Amazonian Ecuador	Coumarins, fraxin, scopoletin, bergamottin, luteolin-7-glucoside, luteolin, quercetin, naringenin, isorhamnetin	Ecuadorian meliponinae and apis honey (Guerrini et al. 2009)

several ulcers and gastrointestinal disorders (Samarakoon et al. 2011; Poorna et al. 2013; Zahin et al. 2010; Kumar et al. 2011). Honey contains antioxidant compounds that are derived from pollen sources (Estevinho et al. 2012) and that can inhibit oxidative reactions (Manyi-Loh et al. 2011; Erejuwa et al. 2012) by increasing total cellular antioxidant capacity and eliminating reactive oxygen species, thereby reducing DNA damage (Erejuwa et al. 2012; Zhou et al. 2012). Quercetin and galangin have shown great antioxidant effects against lipid peroxidation (Lagouri et al. 2014) .

Anti-inflammatory activity

Inflammation commonly occurs during the healing process. The opening of a capillary and the movement of plasma to an infected wound results in edema. Mild inflammation is considered normal during the healing process; however, it is harmful (Aljadi and Yusoff 2003), triggering leukocyte activity (Van den Berg et al. 2008), and leading to the production of free radicals. The polyphenols present in honey have an oxidizing ability (Shafin et al. 2014), which confers a corresponding anti-inflammatory effect by inhibiting the production of nitric oxide. Flavones (naringenin), isoflavones (daidzein and genistein), and flavonols (isorhamnetin, kaempferol, and quercetin) are classes of polyphenols that inhibit inducible nitric oxide synthase (iNOS) and nuclear factor-κB (NF-κB). Additionally, genistein, kaempferol, quercetin, and daidzein impaired the activation of the signal transducer and activator of transcription 1 (STAT-1) (Hämäläinen et al. 2007).

Antineoplastic activity

The bioactive compound eugenol has been studied and has shown its antineoplastic efficacy by inhibiting the proliferation of colon cancer cells and prostate cancer cells (Jaganathan et al. 2011; Samarghandian et al. 2011). Similarly chrysin, a flavone derivative in honey, was reported to have antitumor activity after it successfully eliminated a melanoma cell line (Pichichero et al. 2010; Pichichero et al. 2011). The significant effects of honey against radiation-induced mucositis after head and neck treatment (Khanal et al. 2010), in addition to its effect in reducing Walker tumor (Tomasin and Cintra Gomes-Marcondes 2011), have demonstrated its positive impact in diminishing either benign or malignant tumors in both in vitro and in vivo studies (Swellam et al. 2003). Other subclasses of phenolic acids, namely protocatechuic and p-hydroxybenzoic acids, have demonstrated significant inhibition toward the growth of prostate cancer (PC-3) and breast cancer (MCF-7) cells (Spilioti et al. 2014).

Antiulcer activity

Derivatives of flavonoids, such as kaempferol, quercetin, hesperetin, and naringin, possess antiulcer efficacy for the treatment of the gastric mucosa (Ghaffari et al. 2012), duodenal ulcers (Gadkari and Balaraman 2015), and gastritis (Suprijono et al. 2011). Their antiulcer activity manifests as the slowing down or hindrance of lipid peroxidation, the neutralization or reduction of cell proliferation, and an increased susceptibility to apoptosis (Almasaudi et al. 2016).

Cardiovascular disease

Polyphenols play a vital role in the treatment and control of cardiovascular diseases (Habauzit and Morand 2011). Quercetin is beneficial for controlling blood pressure (Sánchez-Moreno et al. 2006) and reduces the risk of stroke and coronary heart disease (Galindo et al. 2012). Kaempferol is of great importance in protection against the accumulation of the low-density lipoprotein cholesterol that can cause cardiac disorders. The effective action of polyphenols against cardiovascular diseases is mainly achieved by preventing the oxidization of low-density lipoprotein cholesterol, controlling coronary vasodilatation and reversing platelet clotting in the blood

Table 3 The polyphenols mechanisms of action against human diseases

Mechanism	Polyphenol	Action
Cell cycle arrest at various sequential phases, G0/G1, G1 and G2/ in different cancer cell line (Aliyu et al. 2012) Induces apoptosis and disrupts the mitochondrial membrane potential (Erejuwa et al. 2014) Inhibition of angiogenesis (Fauzi et al. 2011)	Caffeic acid Chrysin Kaempferol Quercetin	Antineoplastic Anti-proliferative Anti-metastatic Anticancer
Inhibition of the expression of pro-inflammatory cytokines (TNF-α, COX-2) (Jaganathan et al. 2011) Suppression of lipopolysaccharide-induced COX-2 expression via the inhibition of nuclear factor for IL-6 DNA-binding activity (Hussein et al. 2012) Inhibition of MMP-9 expression in HaCAT (one among the main mediator responsible for destructive effect in chronic wound) (Woo et al. 2005) Suppression of TNF-α induced MMP-9 expression by modulating AKt signaling in endothelial cells (Majtan et al. 2013)	Apigenin Caffeic acid Chrysin Kaempferol	Anti-inflammatory
Inhibition of acid secretions (Vilegas et al. 1999) Increase the level of mucosal prostaglandins (Majtan et al. 2013)	Kaempferol Myricetin Quercetin	Antiulcer
Sarcoplasmic reticulum calcium release inhibition (de RV et al. 1996) Bind divalent metal ion, thereby preventing free radical formation (Narayana et al. 2001) Inhibition of pro-coagulant activity of adherent white blood cell (Raj and Shalini 1999)	Apigenin, Flavonoids Luteolin	Cardiovascular effects
Formation of complex with proteins, forces as hydrogen bonding, hydrophobic effects, and covalent bond formation (Cowan 1999) Formation of hydrogen bond and stacking of nucleic acid bases, thereby inhibiting DNA and RNA synthesis in bacteria (Kumar and Pandey 2013)	Phenolic acids	Antibacterial
Altering fluid in outer and inner membrane of hydrophilic and hydrophobic regions (Tsuchiya and Iinuma 2000) Inhibition of enzymes related to the and viral polymerase, binding of viral nucleic acid or capsid proteins (Viuda-Martos et al. 2008) Inhibition of viral transcription thereby preventing HIV-1 activation (Critchfield et al. 1996)	Acacetin Apigenin Chrysin Naringenin	Antiviral

TNF-α tumor necrosis factor alpha, *COX-2* cyclooxygenase-2, *IL-6* interleukin 6, *MMP-9* matrix metallopeptidase 9, *HaCAT* immortalized human keratinocyte, *AKt* protein kinase B

stream (Alagwu et al. 2011). Thus, the atherosclerosis that leads to arterial hardening and narrowing is effectively counteracted (Kas'ianenko et al. 2010). Generally, polyphenols with activities against either ordinary or chronic diseases are derived from different plant sources (Gadkari and Balaraman 2015) and thus have different therapeutic mechanisms. Table 3 shows the mechanisms by which polyphenols act against several diseases.

Conclusion

The complex and diverse chemical composition of honey is a prominent key to its broad beneficial activity. However, the polyphenols in honey are not limited to being biomarkers, as they possess direct functions in reducing or terminating the risk of major chronic human diseases, such as cardiovascular disease, cancer, tumors, chronic inflammation, and viral disease. The therapeutic efficacy of honey is mainly derived from the known antioxidant activities of flavonoids or phenolic acids, which naturally exist in small quantities. The isolation and characterization of polyphenols in honey has been a challenging task that has been continuously undertaken by researchers to identify novel bioactive compounds for their future applications in the treatment of human disease.

Acknowledgements
Not applicable.

Funding
Dana Penyelidikan Universiti (UniSZA/2015/DPU/8).

Availability of data and materials
Not applicable.

Authors' contributions
All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher's Note

Author details
[1]School of Biomedicine, Faculty of Health Sciences, Universiti Sultan Zainal Abidin, 21300 Kuala Nerus, Terengganu, Malaysia. [2]Department of Pharmacology, Universiti Sains Malaysia, 16130 Kubang Kerian, Kelantan, Malaysia.

Received: 9 October 2018 Accepted: 3 January 2019
Published online: 16 January 2019

References

Alagwu EA, Okwara JE, Nneli RO, Osim EE (2011) Effect of honey intake on serum cholesterol, triglycerides and lipoprotein levels in albino rats and potential benefits on risks of coronary heart disease. Nig J Physiol Sci 26:161–5

Aliyu M, Odunola OA, Farooq AD, Mesaik AM, Choudhary MI, Fatima B, Erukainure OL (2012) Acacia honey modulates cell cycle progression, pro-inflammatory cytokines and calcium ions secretion in PC-3 cell line. Cancer Science & Theraphy 4(12):401–407

Aljadi AM, Yusoff KM (2003) Isolation and identification of phenolic acids in Malaysian honey with antibacterial properties. Turkish J Med Sci 33:229–236
Allsop KA, Miller JB (1996) Honey revisited: a reappraisal of honey in pre-industrial diets. Br J Nutr 75:513–520
Almasaudi SB, El-Shitany NA, Abbas AT, Abdel-dayem UA, Ali SS, Al Jaouni SK, & Harakeh S (2016) Antioxidant, anti-inflammatory, and antiulcer potential of manuka honey against gastric ulcer in rats. Oxidative medicine and cellular longevity 2016(3643824):10. https://doi.org/10.1155/2016/3643824
Alvarez LM (2011) Honey proteins and their interaction with polyphenols
Alvarez-Suarez JM, Tulipani S, Díaz D et al (2010) Antioxidant and antimicrobial capacity of several monofloral Cuban honeys and their correlation with color, polyphenol content and other chemical compounds. Food Chem Toxicol 48: 2490–2499
Asensio-Ramos M, Hernández-Borges J, Rocco A et al (2009) Food analysis: a continuous challenge for miniaturized separation techniques. J Sep Sci 32: 3764–3800
Baker HG, Baker I (1983) A brief historical review of the chemistry of floral nectar. The biology of nectaries Columbia University Press, New York, pp 126–152
Ball DW (2007) The chemical composition of honey. J Chem Educ 84:1643
Behbahani M (2014) Anti-Hiv-1 activity of eight monofloral Iranian honey types. PLoS One 9:e108195
Bernabé J, Mulero J, Cerdá B et al (2013) Effects of a citrus based juice on biomarkers of oxidative stress in metabolic syndrome patients. J Funct Foods 5:1031–1038
Biesaga M, Pyrzyńska K (2013) Stability of bioactive polyphenols from honey during different extraction methods. Food Chem 136:46–54
Blasco C, Vazquez-Roig P, Onghena M et al (2011) Analysis of insecticides in honey by liquid chromatography–ion trap-mass spectrometry: comparison of different extraction procedures. J Chromatogr A 1218:4892–4901
Brudzynski K, Abubaker K, Miotto D (2012) Unraveling a mechanism of honey antibacterial action: polyphenol/H 2 O 2-induced oxidative effect on bacterial cell growth and on DNA degradation. Food Chem 133:329–336
Brudzynski K, Miotto D (2011) Honey melanoidins: analysis of the compositions of the high molecular weight melanoidins exhibiting radical-scavenging activity. Food Chem 127:1023–1030
Brusotti G, Cesari I, Dentamaro A et al (2014) Isolation and characterization of bioactive compounds from plant resources: the role of analysis in the Ethnopharmacological approach. J Pharm Biomed Anal 87:218–228
Campillo N, Viñas P, Peñalver R et al (2012) Solid-phase microextraction followed by gas chromatography for the speciation of organotin compounds in honey and wine samples: a comparison of atomic emission and mass spectrometry detectors. J Food Compos Anal 25:66–73
Can Z, Yildiz O, Sahin H et al (2015) An investigation of Turkish honeys: their physico-chemical properties, antioxidant capacities and phenolic profiles. Food Chem 180:133–141
Cavalcanti R, Forster-Carneiro T, Gomes M et al (2013) Uses and applications of extracts from natural sources. In: Natural product extraction: principles and applications. R. Soc. Chem, Cambridge, pp 1–3
Chaturvedula VSP, Prakash I (2011) The aroma, taste, color and bioactive constituents of tea. J Med Plant Res 5:2110–2124
Chiang LC, Ng LT, Cheng PW et al (2005) Antiviral activities of extracts and selected pure constituents of Ocimum Basilicum. Clin Exp Pharmacol Physiol 32:811–816
Cowan MM (1999) Plant products as antimicrobial agents. Clin Microbiol Rev 12: 564–582
Critchfield JW, BUTERA ST, FOLKS TM (1996) Inhibition of HIV activation in latently infected cells by flavonoid compounds. AIDS Res Hum Retrovir 12:39–46
da Silva IAA, da Silva TMS, Camara CA et al (2013) Phenolic profile, antioxidant activity and palynological analysis of stingless bee honey from Amazonas, northern Brazil. Food Chem 141:3552–3558
Das A, Datta S, Mukherjee S et al (2015) Evaluation of Antioxidative, antibacterial and probiotic growth stimulatory activities of Sesamum indicum honey containing phenolic compounds and Lignans. LWT-Food Sci Technol 61:244–250
de RV S, Somoza B, Ortega T et al (1996) Isolation of vasodilatory active flavonoids from the traditional remedy Satureja obovata. Planta Med 62:272–274
Di Ferdinando M, Brunetti C, Agati G et al (2014) Multiple functions of polyphenols in plants inhabiting unfavorable Mediterranean areas. Environ Exp Bot 103:107–116
Dimitrova B, Gevrenova R, Anklam E (2007) Analysis of phenolic acids in honeys of different floral origin by solid-phase extraction and high-performance liquid chromatography. Phytochem Anal 18:24–32
Erejuwa OO, Sulaiman SA, Ab Wahab MS (2012) Honey: a novel antioxidant. Molecules 17:4400–4423
Erejuwa OO, Sulaiman SA, Wahab MSA (2014) Effects of honey and its mechanisms of action on the development and progression of cancer. Molecules 19:2497–2522
Estevinho L, Pereira AP, Moreira L et al (2008) Antioxidant and antimicrobial effects of phenolic compounds extracts of Northeast Portugal honey. Food Chem Toxicol 46:3774–3779
Estevinho LM, Feás X, Seijas JA et al (2012) Organic honey from Trás-Os-Montes region (Portugal): chemical, palynological, microbiological and bioactive compounds characterization. Food Chem Toxicol 50:258–264
Eteraf-Oskouei T, Najafi M (2013) Traditional and modern uses of natural honey in human diseases: a review. Iran J Basic Med Sci 16:731
Fauzi AN, Norazmi MN, Yaacob NS (2011) Tualang honey induces apoptosis and disrupts the mitochondrial membrane potential of human breast and cervical cancer cell lines. Food Chem Toxicol 49:871–878
Fingleton J, Corin A, Sheahan D et al (2014) Randomised controlled trial of topical Kanuka honey for the treatment of cold sores. Adv Intern Med 1:119–123
Forbes-Hernández TY, Giampieri F, Gasparrini M et al (2014) The effects of bioactive compounds from plant foods on mitochondrial function: a focus on apoptotic mechanisms. Food Chem Toxicol 68:154–182
Gadkari PV, Balaraman M (2015) Catechins: sources, extraction and encapsulation: a review. Food Bioprod Process 93:122–138
Galindo P, Rodriguez-Gómez I, González-Manzano S et al (2012) Glucuronidated quercetin lowers blood pressure in spontaneously hypertensive rats via Deconjugation. PLoS One 7:e32673
Gašić U, Kečkeš S, Dabić D et al (2014) Phenolic profile and antioxidant activity of Serbian polyfloral honeys. Food Chem 145:599–607
Ghaffari A, Somi MH, Safaiyan A et al (2012) Honey and apoptosis in human gastric mucosa. Health Promot Perspect 2:53
Gheldof N, Wang X-H, Engeseth NJ (2002) Identification and quantification of antioxidant components of honeys from various floral sources. J Agric Food Chem 50:5870–5877
Gil MI, Ferreres F, Ortiz A et al (1995) Plant phenolic metabolites and floral origin of rosemary honey. J Agric Food Chem 43:2833–2838
Graham JM (1992) The hive and the honey bee. Dadant & Sons Inc., Hamilton. https://www.cabdirect.org/cabdirect/abstract/19930233028
Guerrini A, Bruni R, Maietti S et al (2009) Ecuadorian stingless bee (Meliponinae) honey: a chemical and functional profile of an ancient health product. Food Chem 114:1413–1420
Habauzit V, Morand C (2011) Evidence for a protective effect of polyphenols-containing foods on cardiovascular health: an update for clinicians. Ther Adv Chronic Dis. https://doi.org/10.1177/2040622311430006
Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, & Moilanen E (2007) Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-κB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-κB activation along with their inhibitory effect on ions expression and no production in activated macrophages. Mediators of inflammation 2007(45673):10. https://doi.org/10.1155/2007/45673
Harborne JB (1989) Methods in plant biochemistry. Volume 1. Plant Phenolics. Academic Press Ltd. Plant Science Laboratories, University of Reading, Whiteknights, Reading. https://www.cabdirect.org/cabdirect/abstract/19910304248
Huang X, Lin J, Yuan D (2011) Simple and sensitive determination of nitroimidazole residues in honey using stir Bar Sorptive extraction with mixed mode monolith followed by liquid chromatography. J Sep Sci 34:2138–2144
Hussein SZ, Mohd Yusoff K, Makpol S, Yusof M, & Anum Y (2012) Gelam honey inhibits the production of proinflammatory, mediators NO, PGE2, TNF-α, and IL-6 in carrageenan-induced acute paw edema in rats. Evidence-Based Complementary and Alternative Medicine 2012(109636):13. https://doi.org/10.1155/2012/109636
Jaganathan SK, Mazumdar A, Mondhe D et al (2011) Apoptotic effect of eugenol in human colon cancer cell lines. Cell Biol Int 35:607–615
Maróstica RM, Junior Leite AV, & Dragano NRV (2010) Supercritical fluid extraction and stabilization of phenolic compounds from natural sources–review (supercritical extraction and stabilization of phenolic compounds). The Open Chemical Engineering Journal 2010(4):51–60
Kas'ianenko V, Komisarenko I, Dubtsova E (2010) Correction of atherogenic dyslipidemia with honey, pollen and bee bread in patients with different body mass. Terapevticheskii arkhiv 83:58–62

Kassim M, Achoui M, Mansor M et al (2010a) The inhibitory effects of Gelam honey and its extracts on nitric oxide and prostaglandin E 2 in inflammatory tissues. Fitoterapia 81:1196–1201
Kassim M, Achoui M, Mustafa MR et al (2010b) Ellagic acid, phenolic acids, and flavonoids in Malaysian honey extracts demonstrate in vitro anti-inflammatory activity. Nutr Res 30:650–659
Kečkeš S, Gašić U, Veličković TĆ et al (2013) The determination of phenolic profiles of Serbian unifloral honeys using ultra-high-performance liquid chromatography/high resolution accurate mass spectrometry. Food Chem 138:32–40
Khalil M, Alam N, Moniruzzaman M et al (2011) Phenolic acid composition and antioxidant properties of Malaysian honeys. J Food Sci 76:C921–C928
Khanal B, Baliga M, Uppal N (2010) Effect of topical honey on limitation of radiation-induced Oral mucositis: an intervention study. Int J Oral Maxillofac Surg 39:1181–1185
Khoddami A, Wilkes MA, Roberts TH (2013) Techniques for analysis of plant phenolic compounds. Molecules 18:2328–2375
Krell R (1996) Value-added products from beekeeping. FAO, Viale delle Terme di Caracalla, Rome. http://www.hunter-valley-amateur-beekeepers.org/wp-content/uploads/2013/04/Value-added-products-from-beekeeping.-.pdf
Kumar M, Kumar S, Kaur S (2011) Investigations on DNA protective and antioxidant potential of chloroform and ethyl acetate fractions of Koelreuteria paniculata Laxm. Afr J Pharm Pharmacol 5:421–427
Kumar S, & Pandey AK (2013) Chemistry and biological activities of flavonoids: an overview. The Scientific World Journal 2013(162750):16. https://doi.org/10.1155/2013/162750
Kumar V, Lemos M, Sharma M et al (2013) Antioxidant and DNA damage protecting activities of Eulophia Nuda Lindl. Free Radicals Antioxid 3:55–60
Kurhade ST, Momin M, Khanekar P et al (2013) Novel biocompatible honey hydrogel wound healing sponge for chronic ulcers. Int J Drug Deliv 5:353
Lachman J, Orsák M, Hejtmánková A et al (2010) Evaluation of antioxidant activity and total phenolics of selected Czech honeys. LWT-Food Sci Technol 43:52–58
Lagouri V, Prasianaki D, Krysta F (2014) Antioxidant properties and phenolic composition of Greek propolis extracts. Int J Food Prop 17:511–522
Lyu S-Y, Rhim J-Y, Park W-B (2005) Antiherpetic activities of flavonoids against herpes simplex virus type 1 (Hsv-1) and type 2 (Hsv-2) in vitro. Arch Pharm Res 28:1293–1301
Macdonald IO, Oludare AS, Olabiyi A (2010) Phytotoxic and anti-microbial activities of flavonoids in Ocimum gratissimum. Life Sci J 7:3
Majtan J, Bohova J, Garcia-Villalba R et al (2013) Fir honeydew honey flavonoids inhibit Tnf-A-induced Mmp-9 expression in human keratinocytes: a new action of honey in wound healing. Arch Dermatol Res 305:619–627
Manyi-Loh CE, Clarke AM, Ndip RN (2011) An overview of honey: therapeutic properties and contribution in nutrition and human health. Afr J Microbiol Res 5:844–852
Mattonai M, Parri E, Querci D et al (2016) Development and validation of an Hplc-dad and Hplc/Esi-Ms 2 method for the determination of polyphenols in monofloral honeys from Tuscany (Italy). Microchem J 126:220–229
McLoone P, Warnock M, Fyfe L (2016) Honey: A realistic antimicrobial for disorders of the skin. Journal of Microbiology, Immunology and Infection, 49(2):161–167
Merken HM, Beecher GR (2000) Measurement of food flavonoids by high-performance liquid chromatography: a review. J Agric Food Chem 48:577–599
Molan PC (1999) The role of honey in the management of wounds. Journal of wound care 8(8), 415–418.
Molan PC (2006) The evidence supporting the use of honey as a wound dressing. Int J Low Extrem Wounds 5:40–54
Moniruzzaman M, Sulaiman SA, Azlan SAM et al (2013) Two-year variations of phenolics, flavonoids and antioxidant contents in acacia honey. Molecules 18:14694–14710
Morand C, Dubray C, Milenkovic D et al (2011) Hesperidin contributes to the vascular protective effects of orange juice: a randomized crossover study in healthy volunteers. Am J Clin Nutr 93:73–80
Mori A, Nishino C, Enoki N et al (1987) Antibacterial activity and mode of action of plant flavonoids against Proteus vulgaris and Staphylococcus aureus. Phytochemistry 26:2231–2234
Naczk M, Shahidi F (2006) Phenolics in cereals, fruits and vegetables: occurrence, extraction and analysis. J Pharm Biomed Anal 41:1523–1542
Nakajima VM, Macedo GA, Macedo JA (2014) Citrus bioactive Phenolics: role in the obesity treatment. LWT-Food Sci Technol 59:1205–1212
Naqvi SAR, Mahmood N, Naz S et al (2013) Antioxidant and antibacterial evaluation of honey bee hive extracts using in vitro models. Mediterr J Nutr Metab 6:247–253
Narayana KR, Reddy MS, Chaluvadi M et al (2001) Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian J Pharm 33:2–16
Nile SH, Park SW (2014) Edible berries: bioactive components and their effect on human health. Nutrition 30:134–144
Onifade A, Jewell A, Ajadi T et al (2013) Effectiveness of a herbal remedy in six HIV patients in Nigeria. J Herbal Med 3:99–103
Pichichero E, Cicconi R, Mattei M et al (2010) Acacia honey and Chrysin reduce proliferation of melanoma cells through alterations in cell cycle progression. Int J Oncol 37:973–981
Pichichero E, Cicconi R, Mattei M et al (2011) Chrysin-induced apoptosis is mediated through P38 and Bax activation in B16-F1 and A375 melanoma cells. Int J Oncol 38:473–483
Poorna CA, Resmi MS, & Soniya EV (2013) In vitro antioxidant analysis and the DNA damage protective activity of Leaf extract of the Excoecaria agallocha Linn Mangrove plant. In Agricultural Chemistry. IntechOpen. https://doi.org/10.5772/55416. Available from: https://www.intechopen.com/books/agricultural-chemistry/in-vitro-antioxidant-analysis-and-the-dna-damage-protective-activity-of-leaf-extract-of-the-excoecar
Pyrzynska K, Biesaga M (2009) Analysis of phenolic acids and flavonoids in honey. TrAC Trends Anal Chem 28:893–902
Raj K, Shalini K (1999) Flavonoids-a review of biological activities
Ramirez-Estrada K, Vidal-Limon H, Hidalgo D et al (2016) Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules 21:182
Reybroeck W, Jacobs FJ, De Brabander HF et al (2010) Transfer of sulfamethazine from contaminated beeswax to honey. J Agric Food Chem 58:7258–7265
Rodriguez-Mateos A, Vauzour D, Krueger CG et al (2014) Bioavailability, bioactivity and impact on health of dietary flavonoids and related compounds: an update. Arch Toxicol 88:1803–1853
Routray W, Orsat V (2012) Microwave-assisted extraction of flavonoids: a review. Food Bioprocess Technol 5:409–424
Samarakoon S, Chandola H, Shukla V (2011) Evaluation of antioxidant potential of Amalakayas Rasayana: a Polyherbal Ayurvedic formulation. Int J Ayurveda Res 2:23
Samarghandian S, Afshari JT, Davoodi S (2011) Chrysin reduces proliferation and induces apoptosis in the human prostate cancer cell line pc-3. Clinics 66:1073–1079
Sánchez-Moreno C, Plaza L, de Ancos B et al (2006) Nutritional characterisation of commercial traditional pasteurised tomato juices: carotenoids, vitamin C and radical-scavenging capacity. Food Chem 98:749–756
Schmidt JO, Bee Products (1996) Chemical Composition and Application in Bee Products; Mizrahi A, Lensky Y, Eds. 1996; pp 15-26. Carl Hayden Bee Research Center USDA-ARS, Tucson
Seeley TD (2009) The wisdom of the hive: the social physiology of honey bee colonies. Harvard University Press
Shafin N, Othman Z, Zakaria R, & Nik Hussain NH (2014) Tualang honey supplementation reduces blood oxidative stress levels/activities in postmenopausal women. ISRN Oxidative Medicine 2014(364836):4. https://doi.org/10.1155/2014/364836
Spilioti E, Jaakkola M, Tolonen T et al (2014) Phenolic acid composition. Antiatherogenic and anticancer potential of honeys derived from various regions in Greece. PLoS One 9:e94860
Sun SY, Jiang WG, Zhao YP (2012) Comparison of aromatic and phenolic compounds in cherry wines with different cherry cultivars by Hs-Spme-Gc-Ms and Hplc. Int J Food Sci Technol 47:100–106
Suprijono A, Trisnadi S, Negara HP (2011) The effect of honey administration on gastrohistopathological image study in male white Wistar rat induced with indomethacin. Sains Medika 3:41–47
Swellam T, Miyanaga N, Onozawa M et al (2003) Antineoplastic activity of honey in an experimental bladder cancer implantation model: in vivo and in vitro studies. Int J Urol 10:213–219
Tenore GC, Ritieni A, Campiglia P et al (2012) Nutraceutical potential of monofloral honeys produced by the Sicilian black honeybees (*Apis mellifera ssp. sicula*). Food Chem Toxicol 50:1955–1961
Tomasin R, Cintra Gomes-Marcondes MC (2011) Oral administration of aloe vera and honey reduces walker tumour growth by decreasing cell proliferation and increasing apoptosis in tumour tissue. Phytother Res 25:619–623

Tsuchiya H, Iinuma M (2000) Reduction of membrane fluidity by antibacterial Sophoraflavanone G isolated from Sophora Exigua. Phytomedicine 7:161–165
Tura D, Robards K (2002) Sample handling strategies for the determination of biophenols in food and plants. J Chromatogr A 975:71–93
Uthurry C, Hevia D, Gomez-Cordoves C (2011) Role of honey polyphenols in health. J ApiProduct ApiMedical Sci 3:141–159
Van den Berg A, Van den Worm E, van Quarles H et al (2008) An in vitro examination of the antioxidant and anti-inflammatory properties of buckwheat honey. J Wound Care 17:172–179
Vilegas W, Sanommiya M, Rastrelli L et al (1999) Isolation and structure elucidation of two new flavonoid glycosides from the infusion of Maytenus aquifolium leaves. Evaluation of the antiulcer activity of the infusion. J Agric Food Chem 47:403–406
Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J et al (2008) Functional properties of honey, Propolis, and Royal Jelly. J Food Sci 73:R117–R124
Wang J, Li QX (2011) Chemical composition, characterization, and differentiation of honey botanical and geographical origins. Adv Food Nutr Res 62:89–137
Woo KJ, Jeong Y-J, Inoue H et al (2005) Chrysin suppresses lipopolysaccharide-induced cyclooxygenase-2 expression through the inhibition of nuclear factor for Il-6 (Nf-Il6) DNA-binding activity. FEBS Lett 579:705–711
Yao L, Jiang Y, D'Arcy B et al (2004) Quantitative high-performance liquid chromatography analyses of flavonoids in Australian eucalyptus honeys. J Agric Food Chem 52:210–214
Zahin M, Ahmad I, Aqil F (2010) Antioxidant and Antimutagenic activity of Carum copticum fruit extracts. Toxicol in Vitro 24:1243–1249
Zhou J, Li P, Cheng N et al (2012) Protective effects of buckwheat honey on DNA damage induced by hydroxyl radicals. Food Chem Toxicol 50:2766–2773